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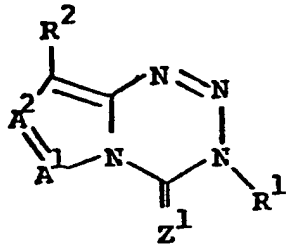
(54) New tetrazine derivatives

(57) Tetrazine derivatives of the general formula:



[wherein R^1 represents cycloalkyl alkyl, alkenyl or alkynyl group, (each alkyl, alkenyl or alkynyl group being unsubstituted or substituted by from 1 to 3 substituents), A^1 represents a nitrogen atom or a group $—CR^3=$ (wherein R^3 represents hydrogen, halogen optionally substituted alkyl or alkenyl, cycloalkyl, cyano, hydroxy, nitro, optionally substituted phenoxy, acyl, alkanoylamino, a sulphide, sulphinyl or sulphonyl group, sulphanoyl group, carbamoyl or thio carbamoyl, when A' represents $—CR^3=$, A^2 represents a nitrogen atom and when A^1 represents a nitrogen atom, A^2 represents a nitrogen atom or a group $—CR^3=$ wherein R^3 is as hereinbefore defined, Z^1 represents an oxygen or sulphur atom, and R^2 represents a sulphide, sulphinyl or sulphonyl group, sulphamoyl, carbamoyl, thio carbamoyl, $CONHNO_2$ or $CSNH \cdot NO_2$ are new therapeutically useful compounds possessing antineoplastic activity.

Processes for the preparation of the tetrazine derivatives are *inter alia* described.

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SPECIFICATION

New tetrazine d rivatives

This invention relates to new tetrazine derivatives, to processes for their preparation and to pharmaceutical compositions containing them.

The compounds of the present invention are the tetrazine derivatives of the general formula shown in Figure I of the drawings assembled at the end of the present specification [wherein R^1 represents a cycloalkyl group, or a straight- or branched-chain alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such alkyl, alkenyl or alkynyl group being unsubstituted or substituted by from one to three substituents selected from halogen (i.e. bromine, iodine or, preferably, chlorine or fluorine) atoms, straight- or branched-chain alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, A^1 represents a nitrogen atom or a group —CR^3= wherein R^3 represents a hydrogen atom or a substituent R^4 wherein R^4 represents a halogen atom, or a straight- or branched-chain alkyl or alkenyl group, containing up to 6 carbon atoms, which may carry up to 3 substituents selected from halogen atoms, optionally substituted phenyl groups, straight- or branched-chain alkoxy, alkylthio and alkylsulphonyl groups containing up to 3 carbon atoms, or R^4 represents a cycloalkyl, cyano, hydroxy, nitro or optionally substituted phenoxy group or a group of the formula —COR^5 (wherein R^5 represents an alkyl or alkoxy group of up to 4 carbon atoms, or a hydroxy group, or an optionally substituted phenyl group) or an alkanoylamino group containing up to 6 carbon atoms, or R^4 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$ or —$CZ^2NR^7R^8$ (wherein *n* represents 0, 1 or 2, R^6 represents a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, a cycloalkyl group or an optionally substituted phenyl group, R^7 and R^8, which may be the same or different, each represents a hydrogen atom or a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, or a cycloalkyl group or an optionally substituted phenyl group or the group —NR^7R^8 represents a heterocyclic group, and Z^2 represents an oxygen or sulphur atom), A^2 represents a nitrogen atom or, when A^1 represents a nitrogen atom, A^2 represents a nitrogen atom or a group —CR^3= wherein R^3 is as hereinbefore defined, Z^1 represents an oxygen or sulphur atom, and R^2 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$, —$CSNR^7R^8$, —$CONR^7R^9$ or —CZ^2NHNO_2 wherein *n*, R^6, R^7, R^8 and Z^2 are as hereinbefore defined, and the group —NR^7R^9 represents a heterocyclic group or R^7 is as hereinbefore defined and R^9 represents a straight- or branched-chain alkyl or alkenyl group containing up to 4 carbon atoms which carries an optionally substituted phenyl substituent, or an optionally substituted phenyl group or, when A^1 represents a nitrogen atom or a group —CR^4= wherein R^4 is as hereinbefore defined and Z^1 and A^2 are as hereinbefore defined or, when A^1 represents a group —CH= and Z^1 represents a sulphur atom and A^2 is as hereinbefore defined, R^2 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$, —$CZ^2NR^7R^8$ or CZ^2NHNO_2 wherein *n*, R^6, R^7, R^8 and Z^2 are as hereinbefore defined] and when R^2 and/or R^3 represents a sulphamoyl or monosubstituted sulphamoyl group and/or R^3 represents a carboxy group, salts thereof, more especially alkali metal, e.g. sodium, salts. Whenever the context so permits reference in this specification to the compounds of the general formula shown in Figure I is meant to include reference to the said salts. The salts are particularly useful as intermediates.

Within the definition of the formula shown in Figure I the optional substituents on the phenyl and phenoxy moieties may be selected from, for example, halogen atoms and alkyl and alkoxy groups containing up to 4 carbon atoms, and nitro groups. Cycloalkyl groups within the definition of the formula shown in Figure I contain 3 to 8, preferably 6, carbon atoms. A heterocyclic group within the definition of the formula shown in Figure I is a 5-, 6- or 7-membered heterocyclic ring which may optionally contain a further heteroatom, i.e. nitrogen, oxygen or sulphur, and which may carry one or two straight- or branched-chain alkyl substituents each containing up to 4 carbon atoms, e.g. a piperidino group or a 2-methyl-, 3-methyl-, 4-methyl-, 2,4-dimethyl-, or 3,5-dimethyl-piperidino group, or a morpholino, pyrrolidin-1-yl, perhydroazepin-1-yl, piperazin-1-yl, 4-methyl-piperazin-1-yl or 1,4-thiazin-4-yl group.

The specifications of British Patent Application No. 2104522 and equivalent applications in other countries which claim priority from original British Patent Application No. 8125791, e.g. United States Patent Application No. 410656, describe compounds of the general formula shown in Figure II of the drawings wherein R^{10} represents a hydrogen atom, a cycloalkyl group or a straight- or branched-chain alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such alkyl, alkenyl or alkynyl group being unsubstituted or substituted by from one to three substituents selected from halogen atoms, straight- or branched-chain alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, and R^{11} represents a carbamoyl group which may carry on the nitrogen atom one or two groups selected from straight- and branched-chain alkyl and alkenyl groups, each containing up to 4 carbon atoms, and cycloalkyl groups.

The said tetrazine derivatives of the formula shown in Figure II possess valuable antineoplastic

also possess valuable immunomodulatory activity and are of use in the treatment of organ grafts and skin grafts and in the treatment of immunological diseases.

The mpounds of the formula shown in Figure I of the present invention possess similar properties with, in certain aspects, an improvement.

The said tetrazine derivativ s of the formula shown in Figure II are useful as intermediates in the preparation of some of the compounds of the formula shown in Figure I of the present invention, for example as described later in this specification.

Particularly important classes of compounds of the formula shown in Figure I include those which exhibit one or more of the following features:—

(i) R^1 represents a methyl or, more especially, a 2-haloethyl group, in particular a 2-chloroethyl group;

(ii) R^2 represents a group of the formula —SOR^6, —SO_2R^6, —$SO_2NR^7R^8$, —$CONR^7R^8$ or —$CONHNO_2$, especially those wherein R^6 represents an alkyl, e.g. methyl, group and those wherein R^7 represents a hydrogen atom or an alkyl, e.g. methyl, group and R^8 represents a hydrogen atom or an alkyl, e.g. methyl, group or a benzyl group optionally substituted by an alkoxy group, e.g. a 4-methoxybenzyl group;

(iii) one of A^1 and A^2 represent a nitrogen atom and the other represents a group —CR^3=;

(iv) R^3 represents a group R^4 wherein R^4 represents an alkyl, e.g. butyl, propyl, ethyl or more particuarly, methyl, group;

(v) A^2 represents a nitrogen atom;

(vi) Z^1 represents an oxygen atom; and/or

(vii) Z^2 represents an oxygen atom.

Important individual compounds of the general formula shown in Figure I include the following:—

8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, A,

8-[*N*-benzyl-*N*-(4-methoxybenzyl)carbamoyl]-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, B,

8-(*N*-benzylcarbamoyl)-3-(2-chloroethyl)'[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, C,

3-(2-chloroethyl)-8-[*N*-(4-methoxybenzyl)-*N*-phenylcarbamoyl]-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, D,

3-(2-chloroethyl)-8-(*N*-phenylcarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, E,

8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, F,

3-(2-chloroethyl)-8-(*N*-methyl-*N*-phenylcarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, G,

8-carbamoyl-3-(2-chloroethyl)-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, H,

3-(2-chloroethyl)-8-(*N*,*N*-dimethylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, I,

3-(2-chloroethyl)-8-(*M*-methylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, J,

3-(2-chloroethyl)-8-methylsulphonyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one, K,

3-methyl-8-methylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, L,

3-(2-chloroethyl)-8-[*N*-(4-methoxybenzyl)-sulphamoyl]-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, M,

3-(2-chloroethyl)-8-sulphamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, N,

8-carbamoyl-3-(2-chloroethyl)-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, O,

8-carbamoyl-3-methyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, P,

3-(2-chloroethyl)-8-piperidinocarbonyl-[3*H*]-imidazolo[5,1-d]-1,2,3,5-tetrazin-4-one, Q,

6-butyl-8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, R,

8-carbamoyl-3-(2-chloroethyl)-6-cyclohexyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, S,

8-carbamoyl-3-(2-chloroethyl)-6-phenethyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, T,

6-benzyl-8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-

8-carbamoyl-3-(2-chloroethyl)-6-isopropyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, V,
8-carbamoyl-3-(2-chl roethyl)-6-propyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, W,
8-carbamoyl-3-(2-chloroethyl)-6-ethyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, X,
3-(2-chloroethyl)-8-(4-methoxybenzyl)sulphamoyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, Y,
3-(2-chloroethyl)-6-methyl-8-sulphamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, Z,
3-(2-chloroethyl)-8-dimethylsulphamoyl-6-methyl-[3*H*]-imidazol[5,1-d]-1,2,3,5-tetrazin-4-one, AA,
3-(2-chloroethyl)-6-methyl-8-methylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, BB,
3-(2-chloroethyl)-8-(dimethylcarbamoyl)-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, CC,
and 3-(2-chloroethyl)-8-(*N*-nitrocarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, DD,
3-methyl-8-methylsulphonyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, EE,
3-(2-chloroethyl)-8-methylsulphonyl-[3*H*]-pyrazolo-[5,1-d]-1,2,3,5-tetrazin-4-one, FF,
3-(2-chloroethyl)-6-methyl-8-methylsulphinyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, GG,
3-(2-chloroethyl)-8-ethylsulphonyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, HH,
and 3-(2-chloroethyl)-6-methyl-8-propylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one. II.

The letters A to II are allocated to the compounds for easy reference later in the specification.

Compounds of particular importance include compounds C, K, L, M, O, Q, R, W, X and DD, more especially compounds I, J, N and BB, and most especially compounds H, Z, AA and CC.

The new tetrazine derivatives of the general formula shown in Figure I have proved particularly active in mice at daily doses between 0.2 and 320 mg/kg animal body weight, administered intraperitoneally, against TLX5 (S) lymphoma according to the procedure of Gescher *et al.*, Biochem. Pharmacol. (1981), *30*, 89, and ADJ/PC6A and M5076 (reticulum cell sarcoma). Against leukaemia L1210, grafted intraperitoneally, intracerebrally and intravenously, and P388, according to the procedure described in "Methods of Development of New Anticancer Drugs" (NCI Monograph 45, March 1977, pages 147—149, National Cancer Institute, Bethesda, United States) the compounds were active both intraperitoneally and orally at doses of between 1 and 320 mg/kg animal body weight. Inhibition of both primary tumour and metastasis was obtained against the Lewis lung carcinoma by similar dosage regimes. Against the B16 melanoma and C38 tumour in mice (NCI Monograph 45, *op, cit*) the compounds were active intraperitoneally at doses of between 6.25 and 40 mg/kg animal body weight. Against colon carcinoma C_{26} in mice, grafted subcutaneously, the compounds were active orally at doses of between 2 and 40 mg/kg animal body weight.

The compounds of the general formula shown in Figure I may be prepared by the application or adaptation of methods known *per se*.

According to a feature of the present invention, the compounds of the general formula shown in Figure I wherein R^2 is other than a sulphamoyl, mono(optionally substituted phenyl)carbamoyl, mono(optionally substituted phenyl)thiocarbamoyl, nitrocarbamoyl or nitrothiocarbamoyl group are prepared by the reaction of a compound of the general formula shown in Figure III of the drawings [wherein A^1 and A^2 are as hereinbefore defined and R^{12} represents a group within the above definition of R^2 other than a sulphamoyl, mono(optionally substituted phenyl)carbamoyl, mono(optionally substituted phenyl)thiocarbamoyl, nitrocarbamoyl or nitrothiocarbamoyl group] with a compound of the general formula:—

$$R^1NCZ^1 \qquad \text{IV}$$

wherein R^1 and Z^1 are as hereinbefore defined. The reaction may be effected in the absence or presence of an anhydrous organic solvent, for example a chlorinat d alkan , e.g. dichloromethane, or ethyl acetate, acetonitrile, *N*-methylpyrrolidin-2-one or hexam thylphosphoramide, at a temperature between 0° and 120°C. The reaction may be continued for up to 30 days. Light should preferably be excluded from the reaction mixture.

According to a further feature of the present invention, compounds of the general formula shown in Figure I wherein R^2 represents a mono(optionally substituted phenyl)carbamoyl or mono(optionally

from corresponding compounds, within the general formula shown in Figure I, wherein R^2 represents a group of the formula $—CZ^2NR^{12}R^{13}$ (wherein R^{12} represents an optionally substituted phenyl group, R^{13} represents an optionally substituted benzyl group and Z^2 is as hereinbefore defined) by the application or adaptation of methods known *per se* for the replacement of optionally substituted benzyl groups by hydrogen atoms. Suitable reaction conditions include, for xample, catalytic hydrogenation (using a catalyst such as palladium on charcoal and in a solvent such as ethyl acetate or dimethylformamide); or when R^{13} represents a substituted benzyl group in which the substituent or at least one of the substituents carried by the benzyl group is an alkoxy (e.g. methoxy) group in the *o*- or *p*-position, preferably by reaction with trifluoroacetic acid, preferably in the presence of anisole, and usually at or near room temperature.

According to a further feature of the present invention, compounds of the general formula shown in Figure I wherein R^2 represents a group of the formula $—CZ^2NHNO_2$ (Z^2, R^1, A^1, A^2 and Z^1 being as hereinbefore defined) are prepared by the nitration of compounds of the general formula shown in Figure V of the drawings wherein R^{14} represents a group of the formula $—CZ^2NH_2$ (Z^2, R^1, A^1, A^2 and Z^1 being as hereinbefore defined). The reaction may be carried out near or below room temperature, preferably between 0° and 10°C, in the presence of a nitrating mixture such as a mixture of concentrated sulphuric acid and concentrated nitric acid.

According to a further feature of the present invention, compounds of the formula shown in Figure I wherein R^1, A^1, A^2, and R^2 are as hereinbefore defined and Z^1 represents a sulphur atom are prepared from compounds of the general formula shown in Figure VI of the drawings (wherein R^1, A^1 and R^2 are as hereinbefore defined) and R^{15} represents a group of the formula $—S(O)_nR^6$, $—SO_2NR^7R^8$, $—CZ^2NR^7R^8$ or $—CZ^2NHNO_2$, R^6, R^7, R^8, *n* and Z^2 being as hereinbefore defined) by the action of phosphorus pentasulphide. The reaction may be carried out in an organic solvent, for example an aromatic solvent such as benzene, toluene or xylene, or in pyridine or a derivative such as lutidine, and preferably at an elevated temperature, for example between 50° and 120°C.

According to a further feature of the present invention, compounds of formula I wherein R^1, A^1, A^2 and Z^1 are as hereinbefore defined and R^2 represents a group of the formula $—CSNR^7R^8$ are prepared from compounds of the formula shown in Figure VII of the drawings wherein R^1, A^1, A^2 and Z^1 are as hereinbefore defined and R^{16} represents a group of the formula $—CONR^7R^8$ (R^7 and R^8 being as hereinbefore defined) by reaction with phosphorus pentasulphide under conditions similar to those described hereinbefore for the reaction of phosphorus pentasulphide with compounds of the formula shown in Figure VI.

The aforementioned salts of certain compounds of the formula shown in Figure I are prepared by the application or adaptation of methods known *per se*, for example by reaction of the parent compound of the formula shown in Figure I with an alkali metal hydroxide, carbonate or, preferably, bicarbonate, in an aqueous or aqueous-organic medium, followed by isolation of the salt by methods known *per se*.

When a mixture of products is obtained in any of the abovementioned processes they may be separated by the application or adaptation of methods known *per se*, e.g. chromatography.

Compounds of the general formula shown in Figure III may be prepared by the application or adaptation of methods known *per se*, for example methods described by Shealy *et al.*, J. Org. Chem. (1961), *26*, 2396, and Cheng *et al.*, J. Pharm. Sci. (1968), *57* 1044, and methods described hereinafter in the Reference Examples.

By the term 'methods known *per se*' as used in the present specification is meant methods heretofore used or described in the literature.

The following Examples illustrate the preparation of compounds of general formula I according to the present invention, and the Reference Examples illustrate the preparation of intermediates.

Example 1
Compound A

Sodium nitrite (0.44 g) was dissolved in aqueous acetic acid (2M; 10 ml) at 0°C and the solution was stirred at 0°C and treated with finely ground 5-amino-imidazole-4-*N*-benzyl-*N*-phenylcarboxamide hydrochloride (0.7 g; prepared as described in Reference Example 1) in small portions. After 10 minutes the resulting gummy solid was extracted with ethyl acetate (2×20 ml). The combined ethyl acetate extracts wer washed with water and dried over magnesium sulphate.

The resulting solution of 5-diazoimidazole-4-*N*-benzyl-*N*-phenylcarboxamid was treated with methyl isocyanate (5 ml) and the mixture was stirred at room temperature in the dark for 24 hours. The solution was then evaporated to low volume and the residue was subjected to medium pressure column chromatography, luting with ethyl acetate, to give 8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.22 g) in the form of a white, crystalline solid, m.p. 168—170°C (with decomposition) [Elemental analysis: C, 62.6; H, 4.41; N, 22.3%; calculated: C, 63.32; H. 4.47; N, 23.32%; I.R. (KBr disc): 3100, 1735, 1620 cm^{-1}; NMR (in DMSO-d_6):— singlets at

Example 2
Compound B

Sodium nitrite (3.7 g) was dissolv d in aqueous acetic acid (2M; 35 ml) at 0°C and the solution was stirred at 0°C and treated with a solution of 5-aminoimidazole-4-*N*-benzyl-*N*-(4-methoxybenzyl)carboxamide hydrochloride (2.2 g; prepared as described in Reference Example 2) in 1,2-dimethoxyethane (10 ml), dropwise. A reddish gum separated which was extracted with ethyl acetate (2x20 ml). The combined ethyl acetate extracts were washed with water and with saturated aqueous sodium chloride solution, and dried over sodium sulphate.

The resulting solution of 5-diazoimidazole-4-*N*-benzyl-*N*-(4-methoxybenzyl)carboxamide was treated with 2-chloroethyl isocyanate (2 ml) and the mixture was allowed to stand at room temperature in the dark for 24 hours. The solution was then evaporated to low volume and the residue was subjected to medium pressure column chromatography, eluting with ethyl acetate, to give crude 8-[*N*-benzyl-*N*-(4-methoxybenzyl)-carbamoyl]-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.5 g) in the form of a brown oil.

Example 3
Compound C

8-[*N*-Benzyl-*N*-(4-methoxybenzyl)carbamoyl]-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.5 g; crude material prepared as described in Example 2) and anisol) (0.5 ml) were dissolved together in trifluoroacetic acid (20 ml) and allowed to stand at room temperature for 18 hours. The mixture was then evaporated to dryness and the residue was subjected to medium pressure column chromatography, eluting with a mixture (2:1 v/v) of ethyl acetate and petroleum ether (b.p. 60°—80°C), to give 8-(*N*-benzylcarbamoyl)-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.34 g), in the form of a colourless solid, m.p. 153—155°C (recrystallised from diethyl ether). [Elemental analysis: C, 49.9; H, 3.92; N, 24.4; Cl, 10.4%; calculated: C, 50.53; H, 3.94; N, 25.26; Cl, 10.65%; I.R. (KBr disc) 3370, 3150, 1755 and 1660 cm^{-1}; NMR (in DMSO-d_6): singlets at 7.3 and 8.9 ppm, doublet at 4.4 ppm and triplets at 4.0, 4.6 and 9.05 ppm].

Example 4
Compound D

Sodium nitrite (0.61 g) was dissolved in water (10 ml) and the solution was stirred at 0°C and treated with a solution of crude 5-aminoimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide (2.5 g; prepared as described in Reference Example 3) in hydrochloric acid (2M; 9 ml) and 1,2-dimethoxyethane (15 ml), dropwise. After 20 minutes the solution was extracted with ethyl acetate (3x50 ml) and the combined extracts were dried over magnesium sulphate and evaporated, to give 5-diazoimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide (2.8 g), in the form of an orange oil.

This oil was dissolved in ethyl acetate (40 ml) and treated with 2-chloroethyl isocyanate (8 ml). The mixture was allowed to stand in the dark for 5 days. The solution was evaporated to low volume and the resulting residue was subjected to medium pressure column chromatography, eluting with ethyl acetate, to give 3-(2-chloroethyl)-8-[*N*-(4-methoxybenzyl)-*N*-phenylcarbamoyl]-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.5 g) in the form of a glass, m.p. 55°C [NMR (in DMSO-d_6):— singlets at 3.6, 5.0 and 8.5 ppm triplets centered at 3.9 and 4.5 ppm, multiplet at 6.6—7.2 ppm; I.R. (KBr disc) 1740 and 1640 cm^{-1}].

Example 5
Compound E

3-(2-Chloroethyl)-8-[*N*-(4-methoxybenzyl)-*N*-phenylcarbamoyl]-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.0 g; prepared as described in Example 4) and anisole (0.2 ml) were dissolved together in trifluoroacetic acid (10 ml) and the solution was allowed to stand at room temperature for 18 hours. The mixture was then evaporated to dryness and the residue was triturated with diethyl ether, to give 3-(2-chloroethyl)-8-(*N*-phenylcarbamoyl)-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.43 g), in the form of a tan solid, m.p. 166°C (with decomposition) (after recrystallisation from ethyl acetate) [Elemental analysis: C, 48.4; H, 3.22; N, 26.0%; calculated: C, 48.99; H, 3.48; N, 26.37%; I.R. (KBr disc): 3390, 1735 and 1680 cm^{-1}; NMR (in DMSO-d_6):— singlets at 8.9 and 10.3 ppm, doublet centred at 7.8 ppm, triplets centred at 4.0 and 4.6 ppm, multiplet at 7.0—7.9 ppm].

Exampl 6
Compound F

Sodium nitrite (2.8 g) was dissolved in aqueous acetic acid (2M; 84 ml) and the solution was stirred at 0°C and treated with finely ground 5-aminoimidazole-4-*N*-benzyl-*N*-phenylcarboxamide hydrochloride (2.8 g; prepared as described in Reference Example 1) in small portions. After 10 minutes the resulting gummy solid was extracted with ethyl acetate (3x30 ml) and the combined extracts were washed with water, and then with saturated aqueous sodium chloride solution, and then dried over magnesium sulphate.

chloroethyl isocyanate (9 ml) and the mixture was allowed to stand in the dark at room temperature for 4 days. The solution was then evaporated to low volume and the residue was subjected to medium pressure column chromatography, eluting with ethyl acetate, to give 8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.0 g) in the form of a glass [Elemental analysis: C, 59.3; H, 4.57; N, 19.9; Cl, 8.5%; calculated: C, 58.75; H, 4.19; N, 20.56; Cl. 8.67%; I.R. (KBr disc): 1740 and 1640 cm^{-1}; NMR (in DMSO-d_6):— singlets at 5.2, 7.1, 7.3 and 8.6 ppm, triplets centred at 4.0 and 4.6 ppm].

Example 7
Compound G

A solution of sodium nitrite (11 g) in water (50 ml) was cooled to 0°C and treated with a solution of 5-aminoimidazole-4-*N*-methyl-*N*-phenylcarboxamide hydrochloride (4.0 g; prepared as described in Reference Example 4) in aqueous acetic acid solution (2M; 40 ml), dropwise. After 10 minutes the resulting mixture was extracted with ethyl acetate (4x100 ml), and the combined extracts were filtered, and dried over magnesium sulphate.

The resulting solution of 5-diazoimidazole-4-*N*-methyl-*N*-phenylcarboxamide was treated with 2-chloroethyl isocyanate (11 ml) and the mixture was allowed to stand in the dark at room temperature overnight. The solution was then evaporated to low volume and the residue was subjected to medium pressure column chromatography, eluting with ethyl acetate, to give a solid (5.75 g). This solid was triturated with diisopropyl ether, and then with dichloromethane. The insoluble residue was recrystallised from a mixture of petroleum ether (b.p. 60°—80°C) and ethyl acetate and then from a mixture of ethyl acetate and diisopropyl ether, to give 3-(2-chloroethyl)-8-(*N*-methyl-*N*-phenylcarbamoyl)-[3*H*]imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.8 g) in the form of a colourless solid, m.p. 130—132° [Elemental analysis C, 50.4; H, 3.91; N, 24.9; Cl, 10.6%; calculated: C,50.53; H, 3.94; N, 25.26; Cl, 10.65%; I.R. (KBr disc): 1750 and 1640 cm^{-1}; NMR (in DMSO-d_6):— singlets at 3.4 7.2 and 8.65 ppm, triplets centred at 3.95 and 4.6 ppm].

Example 8
Compound H

A stirred suspension of 5-diazo-2-methylimidazole-4-carboxamide (1.54 g; prepared as described in Reference Example 5) in ethyl acetate (45 ml) was treated with 2-chloroethyl isocyanate (6.33 g) and the mixture was stirred at ambient temperature for 5 days in the dark. The mixture was then diluted with diethyl ether and the resulting solid was filtered off, washed with diethyl ether and dried *in vacuo* at ambient temperature, to give 8-carbamoyl-3-(2-chloroethyl)-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (2.00 g), m.p. 170°C (with decomposition) [Elemental analysis: C, 37.0; H, 3.49; N, 32.8; Cl, 13.3%; calculated: C, 37.44; H, 3.54; N, 32.75; Cl, 13.82%].

Example 9
Compound I

A solution of 5-diazoimidazole-4-(*N,N*-dimethylsulphonamide) (0.55 g; prepared as described in Reference Example 6) in dry ethyl acetate (40 ml) was treated with 2-chloroethyl isocyanate (3 ml) and the mixture was stirred in the dark for 48 hours. The mixture was then evaporated *in vacuo* at below 40°C to about 15 ml volume and diluted with dry diethyl ether. The resulting solid was filtered off, to give 3-(2-chloroethyl)-8-(*N,N*-dimethylsulphamoyl)-[3*H*]imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.68 g) in the form of greyish needles, m.p. 155—156°C. [Elemental analysis: C, 30.4; H, 3.35; N, 26.8; Cl, 11.8%; calculated: C, 31.3; H, 3.62; N, 27.4; Cl, 11.6%; I.R. 1755 cm^{-1}; NMR (in DMSO-d_6): singlets at 2.80, 8.90 ppm, triplets at 3.99, 4.62 ppm].

Example 10
Compound J

A suspension of 5-diazoimidazole-4-(*N*-methylsulphonamide) (0.7 g; prepared as described in Reference Example 7) in ethyl acetate (40 ml) was treated with 2-chloroethyl isocyanate (3 ml) and the mixture was stirred in a stoppered flask in the dark for 48 hours. The mixture was then evaporated *in vacuo* at below 35°C to approximately half its volume, and was diluted with diethyl ether. The resulting solid was filter d off and washed with diethyl ether, t giv 3-(2-chl roethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-imidazo-[5,1-d]-1,2,3,5-t trazin-4-one (0.32 g), in the form of shining buff plates, m.p. 147—148°C [Elemental analysis: C, 28.5; H, 2.90; N, 28.7%; calculated: C, 28.7; H, 3.08; N, 28.7%; I.R. 1745 cm^{-1}; NMR (in DMSO-d_6): doublet at 2.58 ppm, triplets at 3.98, 4.61 ppm, quart t at 7.94 ppm, singlet at 8.84 ppm].

Exampl 11
Compound K

A solution of 5-diazo-4-methylsulphonylimidazole (0.65 g; prepared as described in Reference Example 8) in dry ethyl acetate (50 ml) was treated with 2-chloroethyl isocyanate (3 ml) and the

vacuo and the oily residue was triturated with petroleum ether (b.p. 60—80°C). The resulting solid was filtered off and subjected to medium pressure chromatography, eluting with ethyl acetate, and the white solid product was triturated with petroleum ether and filtered off, to give 3-(2-chloroethyl)-8-methylsulphonyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.72 g), m.p. 154—155°C. [El mental Elemental analysis: C, 30.3; H, 2.85; N, 25.0; Cl, 11.5%; calculat d: C, 30.28; H, 2.90; N, 25.22; Cl, 11.55%].

Example 12
Compound L

A solution of 5-diazo-4-methylsulphonylimidazole (0.65 g; prepared as described in Reference Example 8) in dry ethyl acetate (60 ml) was treated with methyl isocyanate (3.5 ml) and was left to stand at room temperature in the dark for 3 days. A further quantity of methyl isocyanate (3.5 ml) was added and the mixture was warmed at 40°C for 2 days and then was left to stand at room temperature for 3 days. The mixture was then evaporated *in vacuo* to a volume of between 10 and 15 ml, and was subjected to medium pressure chromatography, eluting with ethyl acetate, to give 3-methyl-8-methylsulphonyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.17 g) in the form of a white crystalline solid, m.p. 185—186°C (with decomposition). [Elemental analysis: C, 31.2; H, 2.89; N, 30.3%; calculated: C, 31.44; H, 3.08; N, 30.56%].

Example 13
Compound M

A solution of 5-diazoimidazole-4-[*N*-(4-methoxybenzyl)sulphonamide] (0.3 g; prepared as described in Reference Example 9) in dry ethyl acetate (25 ml) was treated with 2-chloroethyl isocyanate (1.5 g) and the mixture was stirred at room temperature for 48 hours. The resulting dark solution was filtered and evaporated to dryness. The resulting brown solid was triturated with petroleum ether, filtered off and subjected to medium pressure chromatography, eluting with ethyl acetate, to give a white solid that was triturated with petroleum ether, filtered off and dried at 70°C/10 mm Hg for 1 hour, to give 3-(2-chloroethyl)-8-[*N*-(4-methoxybenzyl)sulphamoyl]-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.2 g), m.p. 155—156°C (with decomposition) [I.R. 1745 cm^{-1}; Elemental analysis: C, 41.9; H, 3.72; N, 20.5%; calculated: C, 42.16; H, 3.79; N, 21.07%].

Example 14
Compound N

3-(2-Chloroethyl)-8-[*N*-(4-methoxybenzyl)sulphamoyl]-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.1 g; prepared as described in Example 13) was dissolved in trifluoroacetic acid (1.0 ml) and anisole (3 drops) and the solution was allowed to stand at room temperature for two hours. The mixture was then evaporated *in vacuo* and the residue was triturated with diethyl ether, to give a yellow solid, which was subjected to medium pressure chromatography, using a mixture of petroleum ether (b.p. 60—80°C) and ethyl acetate (1:1 v/v) as eluent, to give 3-(2-chloroethyl)-8-sulphamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (50 mg), in the form of a white solid, m.p. 183°C (with decomposition) [I.R. 1750 cm^{-1}; NMR (in DMSO-d_6): singlets at 8.8, 7.8 ppm, triplets at 4.58, 3.95 ppm].

Example 15
Compound O

A stirred suspension of 3-diazopyrazole-4-carboxamide (5.9 g; prepared as described by Cheng *et al., op. cit.*) in ethyl acetate (150 ml) was treated with 2-chloroethyl isocyanate (24 ml) and stirred at ambient temperature for 7 days in the dark. The mixture was diluted with diethyl ether and the resulting solid was filtered off and washed with diethyl ether, to give a mixture in the form of a cream solid (8.36 g), m.p. 173—174°C (with decomposition).

A solution of a sample of the said mixture (1.0 g) in dimethyl sulphoxide (20 ml) was heated at 60°C overnight. The solution was then evaporated to dryness (at below 60°C and at pressures down to 0.1 mmHg) and the residue was triturated with a mixture of dichloromethane and diethyl ether. The resulting solid was collected and dissolved in boiling acetonitrile (approximately 50 ml). The resulting solution was treated with deactivated silica gel (3 g containing 20% water) and the mixture was evaporated to dryness. The residue was loaded onto a column of silica gel and subjected to medium pressure chromatography, eluting with ethyl acetate, and recrystallising the product from acetonitrile, to give 8-carbamoyl-3-(2-chloroethyl)-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one (0.25 g), in the form of colourless needles, m.p. 203—204°C (with decomposition) [Elemental analysis: C, 34.8; H, 2.92; N, 34.7; Cl, 14.6%; calculated: C, 34.65; H, 2.91; N, 34.64; Cl, 14.61%].

Exampl 16
Compound P

A stirred suspension of 3-diazopyrazole-4-carboxamide (1.6 g; prepared as described by Cheng *et al., op. cit.*) in dichloromethane (49 ml) and *N*-methylpyrrolid-2-one (2.5 ml) was treated with methyl

the resulting solid was filtered off, to give a mixture in th form of a cream solid (2.24 g), m.p. 179—181°C (with decomposition).

A solution of a sample of this solid (1.0 g) in dimethyl sulphoxide (10 ml) was tr ated with deactivated silica gel (8 g; containing 20% water) and the mixture was evaporated to dryness (at 60°C/0.1 mmHg). The residue was loaded onto the top of a column of silica gel and subjected to medium pressure chromatography, eluting with ethyl acetate. The product was triturated with a small amount of saturated aqueous sodium bicarbonate solution and quickly filtered, to give 8-carbamoyl-3-methyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one (41 mg), in the form of a colourless solid, m.p. 170°C (with decomposition). [NMR (in DMSO-d_6): singlets at 3.95, 7.45, 7.55, 8.50 ppm; I.R. (KBr disc): 3400, 3160, 1750 and 1680 cm^{-1}].

Example 17
Compound Q

A solution of sodium nitrite (0.79 g) in water (6 ml) was treated with a solution of crude 4-amino-5-piperidinocarbonylimidazole hydrochloride (2.1 g; prepared as described in Reference Example 10) in aqueous acetic acid (1 M; 17 ml), dropwise with stirring, at 5—10°C during 5 minutes. The solution was extracted with ethyl acetate (4x45 ml) and the combined extracts were dried over magnesium sulphate and evaporated at 30°C/0.1 mmHg. The residue was dried in a desiccator over phosphorus pentoxide for 45 minutes, to give 4-diazo-5-piperidinocarbonylimidazole (1.73 g) in the form of red crystals, pure enough for use in the next stage.

A solution of crude 4-diazo-5-piperidinocarbonylimidazole (1.73 g; prepared as described above) in dry ethyl acetate (53 ml) was treated with 2-chloroethyl isocyanate (5.9 ml) and the mixture was stirred in the dark for 2 days. The solution was then evaporated at 30°C/0.1 mmHg and the residue was subjected twice to medium pressure chromatography on silica gel, eluting with a mixture of ethyl acetate and acetonitrile (88:12 v/v). The appropriate fractions were combined and evaporated and the residue was triturated with petroleum ether (b.p. 40—60°C), to give 3-(2-chloroethyl)-8-piperidinocarbonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.46 g) in the form of light purple crystals, m.p. 92—94°C [Elemental analysis: C, 45.3; H, 4.98; N, 26.1%; calculated: C, 46.4; H, 4.87; N, 27.1%; NMR (in DMSO-d_6): singlet at 8.7 ppm, triplets at 4.6 and 4.0 ppm, multiplets at 3.2—3.4 and 1.5—1.8 ppm; I.R. (KBr disc): 1750, 1630 cm^{-1}].

Example 18
Compound R

A stirred solution of sodium nitrite (1.61 g) in water (5 ml) was cooled and maintained at 5—10°C and treated dropwise with a solution of 5-amino-2-butylimidazole-4-carboxamide hydrochloride (1.61 g; prepared as described in West German Patent Specification No. 2358509) in hydrochloric acid (1M; 17.7 ml) during 5 minutes to give a yellow precipitate, which was filtered off and dried in a desiccator over phosphorus pentoxide, to give 2-butyl-5-diazoimidazole-4-carboxamide (0.47 g) in the form of a yellow solid, m.p. 109—111°C (with decomposition), pure enough for use in the next stage.

A solution of crude 2-butyl-5-diazoimidazole-4-carboxamide (0.47 g; prepared as described above) in ethyl acetate (14 ml) was treated with 2-chloroethyl isocyanate (1.5 ml) and left to stand in the dark for 24 hours. The resulting fawn solid was filtered off and recrystallised from a mixture of ethyl acetate and acetonitrile, to give 6-butyl-8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.49 g), in the form of colourless crystals, m.p. 165—167°C (with decomposition) [Elemental analysis: C, 43.9; H, 4.90; N, 27.9; Cl, 12.0%; calculated: C,44.2: H, 5.06; N, 28.1; Cl, 11.9%].

Example 19
Compound S

A stirred solution of sodium nitrite (0.44 g) in water (3.7 ml) was cooled and maintained at 5—10°C and treated dropwise with a solution of 5-amino-2-cyclohexylimidazole-4-carboxamide hydrochloride (1.1 g; prepared as described in West German Patent Specification No. 2358509) in aqueous acetic acid (2M; 28 ml) during 5 minutes. The resulting orange precipitate was filtered off and dried in a desiccator ov r phosphorus pentoxide for 1 hour, to give crude 2-cyclohexyl-5-diazoimidazol -4-carboxamide (0.86 g), in the form of an orange solid, pure enough for use in the next stage.

A solution of the crude 2-cyclohexyl-5-diazoimidazole-4-carboxamide (0.86 g; prepared as described above) in ethyl acetate (17 ml) was treated with 2-chloroethyl isocyanate (2.0 ml) and left to stand in the dark for 24 hours. The resulting solid was filtered off and recrystallised from ethyl acetate, to give 8-carbamoyl-3-(2-chloro thyl)-6-cyclohexyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.23 g) in the form of colourless crystals, m.p. 245—248°C (with decomposition) [Elemental analysis: C,

Example 20
Compound T

A stirred solution of sodium nitrite (0.58 g) in water (4.7 ml) was cooled and maintained at 5—10°C and treated dropwise with a solution of 5-amino-2-phenethylimidazole-4-carboxamide hydrochloride (1.8 g; prepared as described in Reference Example 11) in aqueous acetic acid (2M; 18 ml) during 5 minutes. The resulting yellow precipitate was filtered off and dried in a desiccator over phosphorus pentoxide for 1 hour, to give crude 5-diazo-2-phenethylimidazole-4-carboxamide (2.0 g) in the form of a yellow solid, pure enough for use in the next stage.

A suspension of crude 5-diazo-2-phenethylimidazole-4-carboxamide (2.0 g; prepared as described above) in ethyl acetate (29 ml) was treated with 2-chloroethyl isocyanate (3.4 ml) and stirred in the dark for 24 hours. The resulting solid was filtered off and recrystallised twice from ethyl acetate, to give 8-carbamoyl-3-(2-chloroethyl)-6-phenethyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.44 g), in the form of colourless crystals, m.p. 179—181°C (with decomposition) [Elemental analysis: C, 51.8; H, 4.19; N, 24.2; Cl, 10.2%; calculated: C, 52,0; H, 4.36; N, 24.2; Cl, 10.2%].

Example 21
Compound U

A solution of 2-benzyl-5-diazoimidazole-4-carboxamide (2.4 g; prepared as described in Reference Example 12) in dry ethyl acetate (150 ml) was treated with 2-chloroethyl isocyanate (10 ml) and the reaction mixture was left to stand at room temperature in the dark for 20 hours. The reaction mixture was then evaporated to dryness and the residue was triturated with petroleum ether (b.p. 60—80°C; 2×30 ml) to remove excess 2-chloroethyl isocyanate. The remaining residue was then triturated with dichloromethane (2×50 ml) to extract the desired product from insoluble 1,3-bis(2-chloroethyl)urea byproduct. The combined dichloromethane extracts were evaporated to dryness and subjected to medium pressure chromatography on silica gel, eluting with ethyl acetate. The appropriate fractions were combined, evaporated and recrystallised from ethyl acetate, to give 6-benzyl-8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.7 g), m.p. 161—163°C (with decomposition) [Elemental analysis: C, 50.4; H, 3.96; N, 25.4; Cl, 10.8%; calculated: C, 50.53; H, 3.94; N, 25.26; Cl, 10.66%; I.R. 1740 cm^{-1}].

Example 22
Compound V

A solution of 5-diazo-2-isopropylimidazole-4-carboxamide (1.2 g; prepared as described in Reference Example 13) in dry ethyl acetate (75 ml) was treated with 2-chloroethyl isocyanate (5 ml) and the mixture was left to stand in the dark at room temperature for 5 days. The resulting crystalline solid was filtered off and was washed with petroleum ether (b.p. 60—80°C), to give 8-carbamoyl-3-(2-chloroethyl)-6-isopropyl-[3*H*]-imidazo[5,1-d]-1,2,3;5-tetrazin-4-one (0.2 g), m.p. 189—190°C (with decomposition) [Elemental analysis: C, 42.0; H, 4.64; N, 29.5%; calculated: C, 42.19; H, 4.60; N, 29.5%; I.R. 1740 cm^{-1}].

Example 23
Compound W

A solution of sodium nitrite (0.7 g) in water (6 ml) was added to a solution of 5-amino-2-propylimidazole-4-carboxamide (1.37 g; prepared as described in West German Patent Specification No. 2358509) in aqueous acetic acid (2M; 22 ml) at 0—5°C dropwise, during 5 minutes. The resulting precipitate was filtered off and dried in a desiccator over phosphorus pentoxide, to give 5-diazo-2-propylimidazole-4-carboxamide (0.56 g), in the form of a yellow solid, pure enough for use in the next stage.

A solution of crude 5-diazo-2-propylimidazole-4-carboxamide (0.56 g; prepared as described above) in dry ethyl acetate (14 ml) was treated with 2-chloroethyl isocyanate (1.6 ml) and stirred in the dark for 24 hours. The resulting precipitate was filtered off and washed with ethyl acetate, to give 8-carbamoyl-3-(2-chloroethyl)-6-propyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.48 g), in the form of a buff solid, m.p. 145—148°C (with decomposition) [Elemental analysis: C, 41.1; H, 4.4; N, 28.5%; calculated: C, 42.2; H, 4.60; N, 29.5%; NMR (in DMSO-d_6): singlet at 7.7 ppm; triplets at 4.6, 4.0, 3.2 and 1.0 ppm, multiplet at 1.8 ppm; I.R. (KBr disc): 1750, 1695 cm^{-1}].

Example 24
Comp und X

A stirred solution of sodium nitrite (0.44 g) in water (3.8 ml) was added to a solution of 5-amino-2-ethylimidazole-4-carboxamide (0.80 g; prepared as described in West German Patent Specification No. 2358509) in aqueous acetic acid (2M; 14 ml) at 0—3°C, dropwise, during 5 minutes. The resulting precipitate was filtered off and dried in a desiccator over phosphorus pentoxide for 1 hour, to give 5-diazo-2-ethylimidazole-4-carboxamide (0.62 g) in the form of a yellow solid, m.p. 139°C (with decomposition), pure enough for use in the next stage.

above) in dry ethyl acetate (22 ml) was treated with 2-chloroethyl isocyanate (2.4 ml) and stirred in the dark for 24 hours. Th resulting precipitate was filtered off and washed with ethyl acetate, to give 8-carbamoyl-3-(2-chloroethyl)-6-ethyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.59 g), in the form of pale grey crystals, m.p. 172—174°C (with decomp sition) [Elemental analysis: C, 39.7; H, 3.98; N, 31.0; Cl, 13.1%; calculated: C, 39.9; H, 4.10; N, 31.0; Cl, 13.1].

Example 25
Compound Y

Dry ethyl acetate (100 ml) was treated with 5-diazo-4-(4-methoxybenzyl)sulphamoyl-2-methylimidazole (2.45 g; prepared as described in Reference Example 14), followed by 2-chloroethyl isocyanate (3 ml) and the reaction mixture was stirred in the dark at room temperature for 56 hours. The mixture was then treated with a further quantity of 2-chloroethyl isocyanate (3 ml) and stirred in the dark at room temperature for a further period of 24 hours. The reaction mixture was then evaporated to dryness and the residue was triturated with petroleum ether (b.p. 60—80°C; 3x25 ml) to remove excess 2-chloroethyl isocyanate. The remaining residue was then triturated with dichloromethane (2x50 ml) to extract the desired product from insoluble 1,3-bis(2-chloroethyl)urea byproduct. The combined dichloromethane extracts were evaporated to dryness and subjected to medium pressure chromatography on silica gel, eluting with ethyl acetate, to give 3-(2-chloroethyl)-8-(4-methoxybenzyl)sulphamoyl-6-methyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (1.5 g), m.p. 159—160°C) with decomposition) [I.R. 1760 cm^{-1}].

Example 26
Compound Z

A solution of 3-(2-chloroethyl)-8-(4-methoxybenzyl)sulphamoyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (1.5 g; prepared as described in Example 25) in trifluoroacetic acid (10 ml) and anisole (10 drops) was left to stand at room temperature overnight. The reaction mixture was evaporated *in vacuo* and the residue was triturated with diethyl ether. The resulting pale brown solid was filtered off and recrystallised from acetone, to give 3-(2-chloroethyl)-6-methyl-8-sulphamoyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (0.55 g), m.p. 199—200°C (with decomposition) [Elemental analysis: C, 29.1; H, 3.02; N, 28.8; Cl, 12.1; S, 10.6%; calculated: C, 28.72; H, 3.10; N, 28.71; Cl, 12.11; S, 10.95%; I.R. 1760, 3310 cm^{-1}].

Example 27
Compound AA

A solution of 5-diazo-4-dimethylsulphamoyl-2-methylimidazole (1.8 g; prepared as described in Reference Example 15) in dry ethyl acetate (100 ml) was treated with 2-chloroethyl isocyanate (4 ml) and the mixture was left to stand for 2 days at room temperature. The mixture was then treated with a further quantity of 2-chloroethyl isocyanate (4 ml) and left to stand at room temperature for a further period of 6 days. The reaction mixture was then evaporated *in vacuo* and the residue was triturated with petroleum ether (b.p. 60—80°C; 2x25 ml). The remaining solid was dissolved in ethyl acetate and subjected to medium pressure chromatography on silica gel, eluting with ethyl acetate. The appropriate fractions were combined, evaporated to dryness, and triturated with petroleum ether (b.p. 60—80°C), to give 3-(2-chloroethyl)-8-dimethylsulphamoyl-6-methyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one (2.17 g), m.p. 137—138°C [Elemental analysis: C, 33.8; H, 3.91; N, 25.8; Cl, 11.2; S, 9.7%; calculated: C, 33.7; H, 4.09; N, 26.20; Cl, 11.05; S, 10.0%].

Example 28
Compound BB

A solution of 5-diazo-2-methyl-4-methylsulphonylimidazole (0.4 g; prepared as described in Reference Example 16) in dry ethyl acetate (30 ml) was treated with 2-chloroethyl isocyanate (2 ml) and left to stand at room temperature, in the dark, for 4 days. The mixture was then evaporated to dryness and the residue was triturated with petroleum ether (b.p. 60—80°C; 2x25 ml) to remove excess 2-chloroethyl isocyanate. The remaining residue was dissolved in ethyl acetate and subjected to medium pressure chromatography on silica gel, eluting with ethyl acetate, to give 3-(2-chloroethyl)-6-methyl-8-methylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.22 g), m.p. 149—150°C [Elemental analysis: C, 33.0; H, 3.35; N, 23.8; Cl, 12.7%; S, 10.7%; calculated: C, 32.94; H, 3.46; N, 24.01; Cl, 12.15; S, 10.99%; I.R. 1745 cm^{-1}].

Exampl 29
Compound CC

A suspension of 3-diazopyrazole-4-(*N,N*-dimethylcarboxamide) hydrochloride (0.92 g; prepared as described in Referenc Example 17) in dry dichloromethane (50 ml) was treated with 2-chloroethyl isocyanate (2.5 ml) and the stirred suspension was then treated with 1,8-diazabicyclo[5,4,0]undec-7-ene (0.7 g). The resulting solution was stirred at room temperature in the dark overnight. The

60—80°C). The insoluble residue was subjected to medium pressure chromatography, eluting with ethyl acetate. The appropriate fracti ns were combined, evaporated to dryness and the residue was recrystallised from ethyl acetate, to give 3-(2-chloroethyl)-8-(dimethylcarbamoyl)-[3*H*]-pyrazolo[5,1-d] 1,2,3,5-tetrazin-4-one (0.38 g) in the form of colourless crystals, m.p. 116—118°C (with decomposition) [Elemental analysis: C, 39.7; H, 3.96; N, 30.9; Cl, 13.1%; calculated: C, 39.93; H, 4.10; N, 31.05; Cl, 13.1%; I.R. (KBr disc): 1770, 1630 cm^{-1} NMR (in acetone-d_6): singlets at 3.25 and 8.40 ppm, triplets at 4.25 ppm and 4.95 ppm].

Example 30
Compound DD

Stirred concentrated sulphuric acid (2.5 ml) was treated with 8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.24 g; prepared as described in British Patent Specification No. 2104522). The mixture was cooled to 0°C and treated dropwise with concentrated nitric acid (d=1.42; 1 ml). The solution was maintained at 4°C for 1 hour and then was poured on to ice. The precipitated solid was collected, washed with water, and recrystallised from aqueous acetone, to give 3-(2-chloroethyl)-8-(*N*-nitrocarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one (0.28 g) in the form of colourless crystals, m.p. 160—161°C (with decomposition) [Elemental analysis: C, 28.6; H, 1.89; Cl, 12,0; N, 33.6%; calculated: C, 29.23; H, 2.10; Cl, 12.33; N, 34,09%; I.R. (KBr disc): 3200, 1750, 1720 and 1620 cm^{-1}; NMR (DMSO-d_6): triplets at 4.05 ppm (J=6 Hz) and 4.70 ppm (J=6 Hz), singlet at 9.05 ppm, broad singlet at 8.25 ppm; m/e 287/289 (M^+)].

Example 31
Compounds EE, FF, GG, HH and II

By proceeding in a manner similar to that described in Examples 1, 2, 4, 6 to 13, 15 to 25 and 27 to 29 and using the appropriate diazo compounds as intermediates (prepared by the application or adaptation of methods described in the following Reference Examples), there were prepared:—

3-methyl-8-methylsulphonyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, in the form of a colourless solid, m.p. 182—184°C;

3-(2-chloroethyl)-8-methylsulphonyl-[3*H*]-pyrazolo-[5,1-d]-1,2,3,5-tetrazin-4-one, in the form of a colourless solid, m.p. 166—171°C;

3-(2-chloroethyl)-6-methyl-8-methylsulphinyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, in the form of a yellow solid, m.p. 118—120°C;

3-(2-chloroethyl)-8-ethylsulphonyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, m.p. 146—147°C; and

3-(2-chloroethyl)-6-methyl-8-propylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, in the form of a white crystalline solid, m.p. 85—86°C.

Reference Example 1

(i) An intimate mixture of 5-nitroimidazole-4-carboxylic acid (2.0 g) and phosphorus pentachloride (2.67 g) was stirred and heated in an oil bath at 120°C for 1 hour. The resulting yellow slurry was evaporated at 60°C/0.1 mmHg for 30 minutes, to give 1,6-dinitro-5*H*,10*H*-diimidazo[1,5-a:1′,5′-d]pyrazine-5,10-dione (1.90 g) in the form of a yellow solid, m.p. 249—251°C (with decomposition). [I.R. (KBr disc): 1750 cm^{-1}; m/e 278 (M^+)].

[Windaus, Ber., 1923, *56* 684 and Gireva, Chem. Abs., *59*, 1622e, using the same method, describe their products as "5-nitroimidazole-4-carbonyl chloride"].

(ii) A mixture of 1,6-dinitro-5*H*,10*H*-diimidazo-[1,5-a:1′,5′-d]pyrazine-5,10-dione (5.8 g), *N*-benzylaniline (15 g) and tetrahydrofuran (250 ml) was heated at reflux for 6 hours. The tetrahydrofuran was evaporated off *in vacuo* and the residual gum was partitioned between dilute hydrochloric acid (2N; 1 litre) and ethyl acetate (1 litre). Insoluble *N*-benzylaniline hydrochloride was removed by filtration, and the ethyl acetate layer was separated. The aqueous phase was extracted twice more with ethyl acetate and the combined organic phases were washed with dilute hydrochloric acid (2N), and then with water, dried over magnesium sulphate, and evaporated to dryness to give an orange gum. The gum was triturat d twic with boiling diethyl ether, to give a colourless solid, which was crystallised from isopropanol, to give 5-nitroimidazole-4-*N*-benzyl-*N*-phenylcarboxamide (4.0 g), in the form of colourless flakes, m.p. 237—240°C [Elemental analysis: C, 62.3; H, 4.28; N, 17.3%; calculated: C, 63.35; H; 4.38; N, 17.38%; I.R. (KBr disc): 1665 cm^{-1}].

(iii) A solution of 5-nitroimidazole-4-*N*-benzyl-*N*-phenylcarboxamide (4.0 g) in dry ethanol (450 ml) was hydrogenated at 26°C and 3 atmospheres pressure, using a Raney nickel catalyst. When hydrogen absorption was complete (after 4 hours 50 minutes), the mixture was filtered, treated with concentrated hydrochloric acid (3.6 ml) and evaporated to dryness below 40°C. Trituration of the residue with diethyl ether gave 5-aminomidazole-4-*N*-benzyl-*N*-phenylcarboxamide hydrochloride (3.82 g), in the form of a pale yellow solid, m.p. 190—193°C (with decomposition) [NMR (in DMSO-

Reference Example 2

(i) A mixture of *N*-benzyl-*N*-(4-methoxybenzyl)amine [21.9 g; Annalen, (1931), *490* 189], 1,6-dinitro-5*H*,10*H*-diimidazo[5,1-a:1′,5′-d]pyrazine-5,10-dione (6.7 g; prepared as described in Reference Example 1)) and dry tetrahydrofuran (200 ml) was heated at reflux for 18 hours. The t trahydrofuran was evaporated off *in vacuo* and the residual oily solid was partitioned between dilute hydrochloric acid (2N, 500 ml) and ethyl acetate (500 ml). Insoluble *N*-benzyl-*N*-(4-methoxybenzyl)amine hydrochloride was removed by filtration, and the ethyl acetate layer was separated. The aqueous phase was extracted twice more with ethyl acetate and the combined organic phases were washed with dilute hydrochloric acid (2N), then with water, and then with saturated aqueous sodium chloride solution, and then it was dried over sodium sulphate and evaporated to dryness. The residual gum was subjected to medium pressure column chromatography, eluting with ethyl acetate, to give 5-nitroimidazole-4-*N*-benzyl-*N*-(4-methoxybenzyl)carboxamide (2.9 g) in the form of a tan solid, m.p. 167—170°C [after crystallisation from a mixture of petroleum ether (b.p. 60—80°C) and isopropanol] [Elemental analysis: C, 61.3; H, 4.93; N, 15.3%; calculated: C, 62.29; H, 4.95; N, 15.29%; I.R. (KBr disc): 3100—2800, 1640, 1510, 1450 and 1370 cm^{-1}. NMR (in DMSO-d_6 at 120°C): singlets at 3.76, 4.5, 4.6, 7.3 and 7.8 ppm, doublets centred at 6.85 and 7.2 ppm. (The NMR spectrum at room temperature is complicated because of doubling of signals caused by hindered rotation about the bond linking the amide carbonyl group to the amide nitrogen atom).

(ii) A solution of 5-nitroimidazole-4-*N*-benzyl-*N*-(4-methoxybenzyl)carboxamide (2.2 g) in dry ethanol (200 ml) was hydrogenated at room temperature and 3 atmospheres pressure using a Raney nickel catalyst. When hydrogen absorption was complete (after 2 hours 48 minutes), the mixture was filtered, treated with hydrogen chloride gas, and evaporated to dryness below 40°C. Trituration with a mixture of isopropanol and diethyl ether gave 5-aminoimidazole-4-*N*-benzyl-*N*-(4-methoxybenzyl)carboxamide hydrochloride (2.2 g), in the form of a gummy solid, which decomposed above 70°C [I.R. (KBr disc): 3400—2800, 1640 cm^{-1}; NMR (in methanol-d_4): singlets at 3.7, 4.4, 4.5, 7.2 and 8.3 ppm, doublers centred at 6.7 and 6.9 ppm (signals broadened because of hindered rotation about the bond linking the amide carbonyl group to the amide nitrogen atom).

Reference Example 3

(i) A mixture of 1,6-dinitro-5*H*,10*H*-diimidazo-[1,5-a:1′,5′-d]pyrazine-5,10-dione (5.5 g; prepared as described in Reference Example 1), *N*-(4-methoxybenzyl)aniline [14.5 g; Zechmeister *et al*, Ber., (1930), *63*B, 2883] and tetrahydrofuran (250 ml) was heated at reflux for 12 hours. The tetrahydrofuran was then evaporated off *in vacuo* and the residual dark oil was partitioned between dilute hydrochloric acid (2 M; 1000 ml) and ethyl acetate (1000 ml). The organic layer was separated, washed with water, dried over magnesium sulphate and evaporated to dryness. The resulting solid was triturated with diethyl ether, to give 5-nitroimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide (3.18 g), in the form of a peach-coloured solid, m.p. 212—215°C (after recrystallisation from isopropanol). [Elemental analysis: C, 60.4; H, 4.41; N, 16.0%; calculated: C, 61.37; H, 4.58; N, 15.91%; NMR (in DMSO-d_6): singlets at 3.7, 5.0 and 7.7 ppm, multiplet at 6.7—7.3 ppm; I.R. (KBr disc) 1660 cm^{-1}].

(ii) A solution of 5-nitroimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide (2.1 g) in dry ethanol (200 ml) was hydrogenated at 25°C and 3 atmospheres pressure, using a Raney nickel catalyst. When hydrogen absorption was complete (after 5 hours), the mixture was filtered, and evaporated to dryness. The residue was triturated with diethyl ether, to give 5-aminoimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide (1.9 g), in the form of a gum.

{A portion of this gum was characterised as its picrate:— 5-aminoimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide (0.15 g) was dissolved in dry 1,2-dimethoxyethane (3 ml) and treated with a solution of picric acid (0.25 g) in 1,2-dimethoxyethane (5 ml). The resulting crystals were filtered off and washed with diethyl ether to give 5-aminoimidazole-4-*N*-(4-methoxybenzyl)-*N*-phenylcarboxamide picrate (0.07 g), in the form of a yellow solid, m.p. 207°C (with decomposition) [Elemental analysis: C, 49.1; H, 3.64; N, 16.5%; $C_{24}H_{21}N_7O_9{:}2H_2O$ requires: C, 49.1; H, 4.29; N, 16.69%]}.

Ref r nce Example 4

(i) A mixture of 1,6-dinitro-5*H*,10*H*-diimidazo-[1,5-a:1′,5′-d]pyrazine-5,10-dione (8.08g; prepared as described in Reference Example 1), *N*-methylaniline (12.44 g) and tetrahydrofuran (400 ml) was heated at reflux for 24 hours. The tetrahydrofuran was then vaporated off *in vacuo* and the residual dark solid was treated with boiling diethyl ether. The remaining, undissolved solid was subjected to medium pressure column chromatography, eluting with a mixture of ethyl acetate and methanol (1:1 v/v), to give 5-nitroimidazole-4-*N*-methyl-*N*-phenylcarboxamide (4.68 g), in the form of a white solid, m.p. 193°C [Elemental analysis: C, 52.5; H, 3.95; N, 22.2%; calculated: C, 53.66; H, 4.09; N, 22.76%; I.R. 1660 cm^{-1}].

(ii) A solution of 5-nitroimidazole-4-*N*-methyl-*N*-phenylcarboxamide (1.0 g) in dry ethanol (110 ml) was hydrogenated at 23°C and 3 atmospheres pressure, using a Raney nickel catalyst. When

dry hydrogen chloride gas. The solution was then evaporated to dryness, to give 5-aminoimidazole-4-*N*-methyl-*N*-ph nylcarboxamide hydrochloride (0.9 g), in the form of an off-white solid, m.p. 100°C (with decomposition).

[This compound was characterised as its picrate:— A solution of 5-aminoimidazole-4-*N*-methyl-*N*-phenylcarboxamid hydrochloride (0.25 g) in wat r (2 ml) was treated with a solution of picric acid (0.25 g) in 1,2-dimethoxyethane (2 ml). The precipitate was filtered off and washed with 1,2-dimethoxyethane, to give 5-aminoimidazole-4-*N*-methyl-*N*-phenylcarboxamide picrate (0.12 g), in the form of a yellow solid, m.p. 237—238°C (with decomposition) [Elemental analysis: C, 45.3; H, 3.26; N, 21.8%; calculated: C, 45.85; H, 3.39; N, 22.02%; I.R. (KBr disc): 1640 cm^{-1}]].

Reference Example 5

A stirred solution of sodium nitrite (1.0 g) in water (8 ml) was cooled to 0°C and treated with a solution of 4-amino-2-methylimidazole-5-carboxamide hydrochloride (2.0 g; prepared as described in West German Patent Specification No. 2358509) in aqueous acetic acid (2N; 24 ml), maintaining the temperature at between —2°C and 0°C. When the addition was complete the resulting yellow solid was filtered off and dried *in vacuo* over phosphorus pentoxide, to give 5-diazo-2-methylimidazole-4-carboxamide (1.26 g), m.p. 175°C (explodes).

Reference Example 6

(i) A stirred aqueous solution of dimethylamine (30% w/w; 35 ml), cooled in a cold water bath, was treated with 5-nitroimidazole-4-sulphonyl chloride (4.15 g of damp material, freshly prepared from 3.33 g of 5-nitroimidazole-4-thiol ammonium salt by the method of Fisher *et al.*, Can. J. Chem., *39*, 501), in portions. The mixture was stirred for a further 20 minutes and was then evaporated *in vacuo* at below 40°C to reduce the volume by half. The mixture was then made strongly acidic by treatment with concentrated hydrochloric acid and the resulting pale yellow solid was filtered off and recrystallised from dimethylformamide, to give 5-nitroimidazole-4-(*N,N*-dimethylsulphonamide) (2.73 g), in the form of brownish plates, m.p. 282—283°C (with decomposition) [Elemental analysis: C, 27.3; H, 3.65; N, 25.4; S, 14.2%; calculated: C, 27.3; H, 3.66; N, 25.4; S, 14.6%].

(ii) A solution of 5-nitroimidazole-4-(*N,N*-dimethylsulphonamide) (1.0 g) in dry ethanol (100 ml) was hydrogenated at 24°C and 3 atmospheres using a Raney nickel catalyst. The mixture was then filtered and immediately diluted with diethyl ether (200 ml) and treated with dry hydrogen chloride gas until it was slightly acidic. The mixture was then evaporated *in vacuo* at below 30°C. The residual solid was dissolved in hot dry ethanol (20 ml) and the solution was treated with charcoal, filtered, evaporated to 15 ml volume, treated with dry diethyl ether (60 ml) and allowed to crystallise. The resulting solid was filtered off, to give 5-aminoimidazole-4-(*N,N*-dimethylsulphonamide) hydrochloride (0.8 g) in the form of pinkish-buff needles, m.p. 188—189°C (with decomposition) [Elemental analysis: C, 26.1; H, 4.80; N, 23.6; Cl, 15.9; S, 13.9%; $C_5H_{10}N_4O_2S$:HCl requires C, 26.5; H, 4.85; N, 24.7; Cl, 15.6; S, 14.15%].

(iii) A stirred solution of sodium nitrite (0.31 g) in water (5 ml), cooled in an ice-bath, was treated dropwise with a solution of 5-aminoimidazole-4-(*N,N*-dimethylsulphonamide) hydrochloride (0.7 g) in dilute hydrochloric acid (2N; 3.1 ml). The resulting solid was filtered off and washed with ice-cold water, to give 5-diazoimidazole-4-(*N,N*-dimethylsulphonamide) (0.38 g), m.p. 109°C (with decomposition). [I.R. 2180, 2210 cm^{-1}].

A further portion (0.21 g) of slightly less pure product was obtained by extraction of the aqueous liquors at 0°C with ethyl acetate, drying the extract over magnesium sulphate and evaporation *in vacuo*.

Reference Example 7

(i) A stirred aqueous solution of methylamine (25% w/w; 35 ml), cooled in a cold water-bath, was treated with 5-nitroimidazole-4-sulphonyl chloride (4.15 g of damp material, freshly prepared from 3.33 g of 5-nitroimidazole-4-thiol ammonium salt according to the method of Fisher *et al., op. cit.*), in portions. The mixture was stirred for a further 15 minutes and was then evaporated *in vacuo* at below 40°C to reduce the volume by half. The mixture was then made strongly acidic by treatment with concentrated hydrochloric acid and the resulting solid was filtered off and recrystallised from water, to give 5-nitroimidazole-4-(*N*-methylsulphonamide) (2.07 g), in the form of pale yellow blades, m.p. 260—263°C (with decomposition) [Elemental analysis: C, 23.1; H, 2.87; N, 27.4; S, 15.4%; calculated: C, 23.3; H. 2.93; N, 27.2; S, 15.55%].

(ii) A solution of 5-nitroimidazole-4-(*N*-methylsulphonamid) (1.0 g) in dry ethanol (100 ml) was hydrogenated at 24°C and 3 atmospheres using a Raney nickel catalyst. The mixture was then filtered and imm diately diluted with diethyl ether (200 ml) and treated with dry hydrogen chloride gas until it was slightly acidic. The mixture was then evaporated *in vacuo* at below 30°C. The residual solid was dissolved in a minimum volume of hot ethanol and the solution was treated with charcoal, filtered and diluted with diethyl ether. The resulting solid was filtered off, to give 5-aminoimidazole-4-(*N*-

analysis: C, 22.3; H, 4.13; N, 25.5; Cl, 16.9%; $C_4H_8N_4O_2S$:HCl requires C, 22.6; H, 4.26; N, 26.35; Cl, 16.7%].

(iii) A stirred solution of sodium nitrite (0.285 g) in water (4 ml), cooled in an ice-bath, was treated dropwise with a solution of 5-aminoimidazole-4-(*N*-methylsulphonamide) hydrochloride (0.55 g) in dilute hydrochloric acid (2N; 2.8 ml). The resulting solid was filtered off and washed with ice-cold water, to give 5-diazoimidazole-4-(*N*-methylsulphonamide) (0.36 g), m.p. 150°C (with decomposition). [Elemental analysis: C, 25.2; H, 2.47; N, 37.0% calculated: C, 25.7; H, 2.69; N, 37.4%; I.R. 2210 cm^{-1}].

A further portion (0.07 g) of product was obtained by extraction of the aqueous liquors at 0°C with ethyl acetate, drying the extract over magnesium sulphate and evaporation *in vacuo*.

Reference Example 8

A solution of sodium nitrite (0.5 g) in water (5 ml), maintained at 0°C, was treated dropwise with a solution of 5-amino-4-methylsulphonylimidazole hydrochloride [1.0 g; prepared as described by Bennett *et al.*, J.A.C.S., *79(3)*, 2188—2191, (1957)] in dilute hydrochloric acid (2N; 5 ml). The solution was stirred for a further 15 minutes and was then extracted with ethyl acetate (5×20 ml). The combined extracts were dried over sodium sulphate and evaporated *in vacuo* to leave a yellow oil that crystallised on standing, to give 5-diazo-4-methylsulphonylimidazole (0.74 g), m.p. 128—130°C [I.R. 2125 cm^{-1}].

Reference Example 9

(i) A stirred solution of *p*-methoxybenzylamine (10 g) in water (30 ml) was treated with 5-nitroimidazole-4-sulphonyl chloride (6.8 g of damp material, freshly prepared from 6.0 g of 5-nitroimidazole-4-thiol ammonium salt by the method of Fisher *et al., op. cit.*). The mixture soon set solid, whereupon it was treated with isopropanol (20 ml) and triturated, and allowed to stand overnight. The resulting solid was filtered off, washed with ice-cold water, and then it was suspended in water (150 ml) and treated carefully with dilute hydrochloric acid (2N) until the suspension just attained pH 2. The resulting yellow solid was filtered off and washed with a little ice-cold water to give 5-nitroimidazole-4-[*N*-(4-methoxybenzyl)sulphonamide] (7.42 g), m.p. 269—270°C (with decomposition).

(ii) A solution of 5-nitroimidazole-4-[*N*-(4-methoxybenzyl)sulphonamide] (1.0 g) in dry ethanol (100 ml) was hydrogenated for 6 hours at 3 atmospheres using a Raney nickel catalyst (50%). The catalyst was quickly filtered off with the aid of diatomaceous earth and the filtrate was immediately treated with concentrated hydrochloric acid (20 ml). The mixture was evaporated to dryness and the resulting residue was triturated with diethyl ether. The solid was filtered off and washed with diethyl ether, to give 5-aminoimidazole-4-[*N*-(4-methoxybenzyl)sulphonamide] (0.25 g), m.p. 154—155°C.

(iii) A solution of sodium nitrite (0.14 g) in water (5 ml) was treated dropwise with a solution of 5-aminoimidazole-4-[*N*-(4-methoxybenzyl)sulphonamide] (0.5 g) in dilute hydrochloric acid (2N; 10 ml), maintaining the temperature at 0°C. The mixture was stirred at 0°C for a further 15 minutes and then the solid was filtered off, washed with water and dried over phosphorus pentoxide, to give 5-diazoimidazole-4-[*N*-(4-methoxybenzyl)sulphonamide (0.3 g), m.p. 144—146°C (with decomposition) [I.R. 2180 cm^{-1}].

Reference Example 10

(i) A solution of piperidine (6.4 ml) in dry tetrahydrofuran (92 ml) was treated with 1,6-dinitrodiimidazo[1,5-a:1′,5′-d]pyrazine-5,10-dione (4.59 g; prepared as described in Reference Example 1) and stirred at room temperature for 1 hour. The mixture was then evaporated and the residue was dissolved in dilute hydrochloric acid (2N; 92 ml). The solution was extracted with ethyl acetate (3×200 ml) and the combined extracts were dried over magnesium sulphate and evaporated. The residue was subjected to medium pressure chromatography on silica gel, eluting with a mixture of chloroform and methanol (9:1 v/v). The appropriate fractions were combined and evaporated and the residue was washed with diethyl ether, followed by ethyl acetate, to give 4-nitro-5-piperidinocarbonylimidazole (2.72 g), in the form of a yellow solid, m.p. 149—150°C. [Elemental analysis: C, 48.2; H, 5.33; N, 25.1%; calculated: C, 48.2; H, 5.39; N, 25.0%].

(ii) A solution of 4-nitro-5-piperidinocarbonylimidazole (2.68 g; prepared as described above) in methanol (27 ml) and dimethylformamide (27 ml) was treated with platinum oxide (0.27 g) and the shaken mixture was hydrogenated at room temperature and atmospheric pressure. When hydrogen uptake was complete, the mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in dilute hydrochloric acid (2N; 50 ml), the solution was filtered and the filtrate was evaporated *in vacuo*. The resulting residue was washed with acetone, to give 4-amino-5-piperidinocarbonylimidazole hydrochloride (2.1 g), in the form of a pale green solid, m.p. 175—177°C, pure enough for use in the next stage.

Reference Example 11

(90 ml) was treated with hydrogen chloride gas until saturated (3 hours), and left to stand at ro m temperature for 5 days. The mixture was then treated with diethyl ther and the resulting precipitate was filtered off and washed with diethyl ether, to give S-benzyl 3-phenylpropionothioimidate hydrochloride (9.7 g), in the form of a colourless solid, m.p. 158—160°C, pure enough for use in the next stage.

(ii) A solution of α-amino-α-cyanoacetamide (3.3 g) in ethanol (20 ml) was treated with crude S-benzyl-3-phenylpropionothioimidate hydrochloride (9.7 g; prepared as described above) and the mixture was heated at reflux for 15 minutes. The mixture was cooled and the resulting solid was filtered off and recrystallised from methanol, to give 5-amino-2-phenethylimidazole-4-carboxamide hydrochloride (2.3 g), in the form of colourless crystals, m.p. 270—274°C [Elemental analysis: C, 53.8; H, 5.55; N, 21.1%; $C_{12}H_{14}N_4O$:HCl requires: C, 54.0; H, 5.67; N, 21.0%].

Reference Example 12

A solution of sodium nitrite (0.5 g) in water (15 ml) maintained at 0—5°C was treated dropwise with a solution of 5-amino-2-benzyl-4-carbamoylimidazole (1.26 g; prepared as described in West German Patent Specification No. 2358509) in dilute hydrochloric acid (2N; 15 ml). The mixture was stirred at 0°C for a further period of 30 minutes and the resulting pale yellow solid was filtered off, washed with water and dried in a desiccator over phosphorus pentoxide, to give 2-benzyl-5-diazoimidazole-4-carboxamide (0.8 g), m.p. 121—122°C (with decomposition) [I.R. 2180 cm^{-1}].

Reference Example 13

(i) A solution of isobutyronitrile (6.9 g) and benzyl mercaptan (20 ml) in dry dioxan (100 ml) was treated with dry hydrogen chloride gas for 3 hours at 0—10°C. The mixture was then allowed to warm to room temperature and the vessel was closed and allowed to stand at room temperature for 14 days. The mixture was then poured onto diethyl ether (1 litre) and the resulting white precipitate was filtered off and washed with diethyl ether, to give S-benzyl isobutylthioimidate hydrochloride (20.3 g) m.p. 165—166°C [Elemental analysis: C, 57.1; H, 7.0; N, 5.9; Cl, 15.7; S, 13.9%; $C_{11}H_{15}NS$:HCl requires: C, 57.5; H, 7.02; N, 6.1; Cl, 15.43; S, 13.96%].

(ii) A solution of α-amino-α-cyanoacetamide (5.0 g) and S-benzyl isobutylthioimidate hydrochloride (11.5 g; prepared as described above) in dry 2-ethoxyethanol (150 ml) was heated at reflux for 30 minutes. The solvent was evaporated. The resulting dark oil was triturated with a mixture of chloroform and methanol (4:1 v/v; 100 ml) and the insoluble material was filtered off and discarded. The filtrate was subjected to medium pressure chromatography on silica gel, eluting with a mixture of chloroform and methanol (4:1 v/v). The appropriate fractions (identified with ninhydin spray on a sample, which gave an intense yellowish brown colour) were combined and evaporated, to give 5-amino-2-isopropylimidazole-4-carboxamide hydrochloride (4.44 g) in the form of a gummy solid, pure enough for use in the next stage.

(iii) A solution of sodium nitrite (0.5 g) in water (5 ml) maintained at 0°C was treated dropwise with a solution of crude 5-amino-2-isopropylimidazole-4-carboxamide hydrochloride (1.1 g; prepared as described above) in aqueous acetic acid (2N; 20 ml). The mixture was then stirred for a further period of 5 minutes at 0°C and then was extracted with ethyl acetate (3x20 ml). The combined extracts were dried over magnesium sulphate, concentrated *in vacuo* to a volume of 20 ml, and subjected to medium pressure chromatography on silica gel, eluting with ethyl acetate. The appropriate fractions (identified with 2-naphthol spray on a sample, which gave a deep red colour) were combined and evaporated to dryness, to give 5-diazo-2-isopropylimidazole-4-carboxamide (0.43 g), m.p. 120°C (with decomposition) [I.R. 2170 cm^{-1}].

Reference Example 14

(i) A cooled, stirred solution of 2-methyl-5-nitroimidazole (127 g) in aqueous sodium hydroxide solution (5% w/v; 1500 ml) was treated with bromine (160 g), maintaining the temperature at 15—20°C. The mixture was stirred at room temperature for 5.5 hours and the solid which had precipitated was redissolved by treatment of the mixture with aqueous sodium hydroxide solution (2N). Traces of insoluble material were filtered off and the filtrate was acidified to pH1 by treatment with concentrated hydrochloric acid. The resulting white solid was filtered off, recrystallised from ethanol and dried in a desiccator over phosphorus pentoxide, t give 4-bromo-2-methyl-5-nitroimidazole (151 g) in the form of a white crystalline solid, m.p. 267—268°C.

(ii) A stirred solution of 4-bromo-2-methyl-5-nitroimidazole (20.6 gms; prepared as described above) in aqueous ammonia solution (5N; 180 ml) was warmed at 45° and treated with a steady stream of hydrogen sulphide gas for 40 minutes. A yellow crystalline solid was slowly formed. The mixture was cooled in ice and the solid was filtered off, washed with ice-water, and dried in a desiccator over phosphorus pentoxide, to give 4-mercapto-2-methyl-5-nitroimidazole ammonium salt (14.6 g), darkens without melting below 300°C [Elemental analysis: C, 27.2; H, 4.41; N, 31.6; S, 18.2%; $C_4H_4O_2N_3S$:NH_4 rquires: C, 27.27; H, 4.58; N, 31.8; S, 18.2%].

(iii) An ice-cooled, vigorously stirred solution of 4-mercapto-2-methyl-5-nitroimidazole

treated with chl rine gas until a white solid had been formed. The mixture was stirred for a further period of 30 minutes at 0°C and then the solid was filtered off, washed with water and dried in a desiccator over phosphorus pentoxide, to giv 4-chlorosulphonyl-2-methyl-5-nitroimidazole (3.0 g); m.p. 160—162°C (with decomposition) [Elemental analysis: C, 20.8; H, 1.73; N, 18.3; Cl, 15.7; S, 14.6%; calculated: C, 21.29; H, 1.79; N, 18.63; Cl, 15.72; S, 14.21%].

(iv) A cooled solution of 4-methoxybenzylamine (5.48 g) in dry absolute ethanol (20 ml) was treated with 4-chlorosulphonyl-2-methyl-5-nitroimidazole (2.25 g; prepared as described above) and the mixture was stirred at room temperature for 3 hours. The yellow solid which precipitated was filtered off, washed with ethanol and discarded as 4-methoxybenzylamine hydrochloride. The combined filtrate and washings were evaporated to dryness and the resulting residue was suspended in water (50 ml), acidified to pH 1 by treatment with concentrated hydrochloric acid, and extracted with ethyl acetate (3x20 ml). The combined extracts were dried over magnesium sulphate and evaporated to dryness, to give 4-(4-methoxybenzylsulphamoyl)-2-methyl-5-nitroimidazole (2.77 g), in the form of an off-white solid, m.p. 167—170°C [elemental analysis C, 44.2; H, 4.32; N, 17.2; S, 9.5%; calculated: C, 44.17; H, 4.32; N, 17.17; S, 9.83%].

(v) A solution of 4-(4-methoxybenzylsulphamoyl)-2-methyl-5-nitroimidazole (4.5 g; prepared as described above) in dry ethanol (120 ml) was hydrogenated at 3 atmospheres pressure over a Raney nickel catalyst for 30 minutes. The catalyst was filtered off and washed with dry ethanol (10 ml) and the combined filtrate and washings were acidified to pH 1 by treatment with concentrated hydrochloric acid and evaporated to dryness. The resulting residue was triturated with diethyl ether, to give 5-amino-4-(4-methoxybenzylsulphamoyl)-2-methylimidazole hydrochloride (3.25 g); m.p. 183—185°C.

(vi) A solution of sodium nitrite (0.3 g) in water (5 ml) maintained at 0—5°C was treated dropwise with a solution of 5-amino-4-(4-methoxybenzylsulphamoyl)-2-methylimidazole hydrochloride (1.0 g; prepared as described above) in dilute hydrochloric acid (2N; 10 ml). The mixture was stirred for a further period of 5 minutes at 0—5°C and the resulting orange solid was filtered off, washed with water and dried in a desiccator over phosphorus pentoxide, to give 5-diazo-4-(4-methoxybenzylsulphamoyl)-2-methylimidazole (0.75 g), m.p. 140°C (with decomposition) [I.R. 2200 cm^{-1}].

Reference Example 15

(i) An ethanolic solution of dimethylamine (33% w/v; 10 ml) was cooled and stirred and treated portionwise with 4-chlorosulphonyl-2-methyl-5-nitroimidazole (2.25 g; prepared as described in Reference Example 14) and stirred at room temperature for a further period of 45 minutes. The solution was acidified to pH 1 by treatment with concentrated hydrochloric acid and the resulting white solid was filtered off and washed with cold water, to give 4-dimethylsulphamoyl-2-methyl-5-nitroimidazole (1.9 g), m.p. 240—241°C [Elemental analysis: C, 30.5; H, 4.24; N, 23.8; S, 13.8%; calculated: C, 30.77; H, 4.3; N, 23.92; S, 13.69%; NMR (in DMSO-d_6): singlets at 2.30 and 2.80 ppm].

(ii) A solution of 4-dimethylsulphamoyl-2-methyl-5-nitroimidazole (12 g; prepared as described above) in dry ethanol (300 ml) was hydrogenated at 3.5 atmospheres pressure over a Raney nickel catalyst for 1 hour. The catalyst was filtered off and the filtrate was acidified to pH 1 by treatment with concentrated hydrochloric acid and evaporated to dryness. The resulting orange residue was triturated with diethyl ether, to give 5-amino-4-dimethylsulphamoyl-2-methylimidazole (8.9 g), m.p. 215—217°C (with decomposition).

(iii) A solution of sodium nitrite (1.0 g) in water (15 ml) was maintained at 0°C and treated dropwise with a solution of 5-amino-4-dimethylsulphamoyl-2-methylimidazole (2.4 g) in dilute hydrochloric acid (2N; 30 ml), and stirred for a further period of 10 minutes at 0°C. The mixture was extracted with ethyl acetate (5x30 ml) and the combined extracts were dried over magnesium sulphate, evaporated to dryness and triturated with petroleum ether (b.p. 60—80°C), to give 5-diazo-4-dimethylsulphamoyl-2-methylimidazole (1.8 g), m.p. 85—87°C (with decomposition) [I.R. 2180 cm^{-1}].

Reference Example 16

(i) A solution of 4-mercapto-2-methyl-5-nitroimidazole ammonium salt (10.5 g; prepared as described in Reference Example 14) in methanolic sodium methoxide solution [prepared by carefully dissolving sodium (2.3 g) in dry methanol (250 ml)] was treated with methyl iodide (10.7 g) and heated at reflux for 2 hours. The mixture was then evaporated to dryness and the residue was suspended in aqueous sodium hydroxide solution (2N; 100 ml). The suspension was filtered and the filtrate was acidified to pH 1 by treatment with concentrated hydrochloric acid, to give 2-methyl-4-methylthio-5-nitroimidazole (9.0 g), in the form of a yellow solid, m.p. 236—237°C (with decomposition) [Elemental analysis: C, 34.7; H, 4.04; N, 24.3; S, 18.5%; calculated: C, 34.67; H, 4.07; N, 24.26; S, 18.51%].

(ii) A solution of 2-methyl-4-methylthio-5-nitroimidazole (3.46 g; prepared as described above) in glacial acetic acid (35 ml) was heated at 60°C and treated dropwise with aqueous hydrogen peroxide solution (30% w/v; 35 ml). The mixture was then heated at 100°C for 15 minutes, cooled to room temperature and treated with sufficient sodium sulphite to destroy the excess of hydrogen peroxide

continuous liquid-liquid extraction with ethyl acetate for 20 hours. The xtract was evaporated and the remaining white solid was triturated with petroleum ether (b.p. 60—80°C) and filtered off, to give 2-methyl-4-methylsulphonyl-5-nitroimidazole (3.6 g), m.p. 222—224°C [elemental analysis: C, 29.8; H, 3.28; N, 20.6; S, 15.7%; calculated: C, 29.27; H, 3.44; N, 20.48; S, 15.63%].

(iii) A solution of 2-methyl-4-methylsulphonyl-5-nitroimidazol (4.9 g; prepared as described above) in dry ethanol (400 ml) was hydrogenated at 3.5 atmospheres pressure over a Raney nickel catalyst for 30 minutes. The catalyst was filtered off and the filtrate was acidified to pH 1 by treatment with concentrated hydrochloric acid and evaporated to dryness and the resulting residue was triturated with diethyl ether containing a trace of ethanol to give a purple solid which was filtered off and washed with diethyl ether, to give 5-amino-2-methyl-4-methylsulphonylimidazole hydrochloride (4.1 g), m.p. above 300°C [Elemental analysis: C, 27.3; H, 4.51; N, 19.9; Cl, 17.9; S, 13.7%; calculated: C, 28.37; H, 4.76; N, 19.8; Cl, 16.75; S, 15.15%].

(iv) A solution of sodium nitrite (0.25 g) in water (5 ml), maintained at 0°C was treated dropwise with a solution of 5-amino-2-methyl-4-methylsulphonylimidazole hydrochloride (0.53 g; prepared as described above). The mixture was stirred for a further period of 15 minutes at 0°C and was extracted with ethyl acetate (5×15 ml). The combined extracts were dried over magnesium sulphate and evaporated to dryness. The resulting oil was triturated with petroleum ether (b.p. 60—80°C) to give 5-diazo-2-methyl-4-methylsulphonylimidazole (0.4 g), m.p. 100°C (with decomposition) [I.R. 2185 cm^{-1}].

Reference Example 17

(i) A mixture of α-cyano-*N,N*-dimethylacetamide (8.2 g; prepared as described by Bowman *et al.*, J. Chem. Soc., 1954, 1171), acetic anhydride (21 ml) and triethyl orthoformate (21 ml) was heated at 160—170° in a flask fitted with a McIntyre head for 90 minutes, during which time 26 ml of ethyl acetate distillate was collected. The reaction mixture was concentrated *in vacuo* to give a dark oil, which was treated with ethanol (10 ml) and concentrated *in vacuo* again. The residue was distilled at 160—170°C/0.5 mmHg and then subjected to medium pressure chromatography on silica gel, eluting with ethyl acetate, to give 2-cyano-3-ethoxy-*N,N*-dimethylpropenamide (5.3 g) in the form of an off-white, oily solid, pure enough for use in the next stage.

(ii) A solution of crude 2-cyano-3-ethoxy-*N,N*-dimethylpropenamide (5.3 g; prepared as described above) in dry ethanol (50 ml) was treated dropwise with hydrazine hydrate (1.58 g). After the addition was complete the mixture was heated at reflux for 6 hours and then was evaporated to dryness. The residue was substituted to medium pressure chromatography on silica gel, eluting with a mixture of chloroform and methanol (17:3 v/v) and the appropriate fractions were combined and evaporated to dryness. The resulting residue was dissolved in hot isopropanol (5 ml) and treated with concentrated hydrochloric acid (4 ml) and the resulting crystalline precipitate was collected, to give 3-aminopyrazole-4-(*N,N*-dimethylcarboxamide) hydrochloride (1.39 g), in the form of colourless crystals, m.p. 195°C [Elemental analysis: C, 37.8; H, 5.82; N, 29.0; Cl, 18.3%; calculated: C, 37.8; H, 5.82; N, 29.39; Cl, 18.6%; I.R. (KBr disc): 3500, 3400, 3000—2200, 1655 cm^{-1}; NMR (in DMSO-d_6): singlets at 3.0 and 8.1 ppm and broad singlet at 7.2 ppm].

(iii) A saturated solution of dry hydrogen chloride in dry methanol (70 ml) was treated with 3-aminopyrazole-4-(*N,N*-dimethylcarboxamide) hydrochloride (1.39 g; prepared as described above). The stirred mixture was cooled to 0°C and treated with amyl nitrite (2.55 g), dropwise during 15 minutes, maintaining the temperature at 0°C. The resulting solution was left to sand at 2—4°C for 1 hour and was then poured into diethyl ether (800 ml). The resulting solid was collected and washed with diethyl ether, to give 3-diazopyrazole-4-(*N,N*-dimethylcarboxamide) hydrochloride (0.92 g), in the form of colourless crystals, m.p. 150°C (explodes). [Elemental analysis: C, 35.3; H, 3.79; N, 34.3%; $C_6H_7ON_5Cl$; HCl requires: C, 35.74; H, 4.00; N, 34.74%; I.R. (KBr disc): 3000—2100, 2280, 1630 cm^{-1}].

The present invention includes within its scope pharmaceutical compositions which comprise, as active ingredient, at least one compound of the general formula shown in Figure I, together with a pharmaceutical carrier or coating. In clinical practice the compounds of the general formula shown in Figure I will normally be administered orally, rectally, parenterally, for example intraperitoneally or intravenously, e.g. by infusion, or vaginally.

Methods of presentation of pharmaceutically active compounds are well known in the art and a suitable vehicle may be determined by the physician or pharmacist, depending upon such factors as the effect sought, th size, age, sex and condition of the patient and on the properties of the active compound. The compositions may also contain, as is usual in the art, such materials as solid or liquid diluents, wetting agents, preservatives, flavouring and colouring agents and the like.

Solid compositions for oral administration include compressed tablets, pills, dispersible powders, and granules. In such solid compositions one or more of the active compounds is, or are, admixed with at least one inert diluent such as calcium carbonate, potato starch, alginic acid, or lactose. The compositions may also comprise, as is normal practice, additional substances other than inert diluents, e.g. lubricating agents, such as magnesium stearate. Liquid compositions for oral administration

inert diluents commonly used in the art, such as water and liquid paraffin. Besides inert diluents such compositions may also comprise adjuvants, such as wetting and suspending agents, e.g. polyvinylpyrrolidone, and sweetening, flavouring, perfuming and preserving agents. The compositions according to the invention, for oral administration, also include capsules of absorbable material such as gelatin containing one or more of the active substances with or without the addition of diluents or excipients.

Solid compositions for vaginal administration include pessaries formulated in manner known *per se* and containing one or more of the active compounds.

Solid compositions for rectal administration include suppositories formulated in manner known *per se* and containing one or more of the active compounds.

Preparations according to the invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or suspending media are polyethylene glycol, dimethyl sulphoxide, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. These compositions may also include adjuvants such as preserving, wetting, emulsifying and dispersing agents. They may be sterilised, for example, by filtration through a bacteria-retaining filter, by incorporation of sterilising agents in the compositions, or by irradiation. They may also be manufactured in the form of sterile solid compositions, which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

The percentage of active ingredient in the compositions of the invention may be varied, it being necessary that it should constitute a proportion such that a suitable dosage for the therapeutic effect desired shall be obtained. Obviously several unit dosage forms may be administered at about the same time. In general, the preparations should normally contain at least 0.025% by weight of active substance when required for administration by injection, including administration by infusion; for oral administration the preparation will normally contain at least 0.1% by weight of active substance. The dose employed depends upon the desired therapeutic effect, the route of administration and the duration of the treatment.

The tetrazine derivatives of general formula I are useful in the treatment of malignant neoplasms, for example carcinomas, melanomas, sarcomas, lymphomas and leukaemias, at doses which are generally between 0.1 and 200, preferably between 1 and 20, mg/kg body weight per day.

The following Composition Examples illustrate pharmaceutical compositions according to the present invention.

Composition Example 1

A solution suitable for parenteral administration was prepared from the following ingredients:—

8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one	1.0 g
dimethyl sulphoxide	10 ml

by dissolving the 8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one in the dimethyl sulphoxide. The resulting solution was divided, under aseptic conditions, into ampoules at an amount of 1.1 ml per ampoule. The ampoules were sealed, to give 10 ampoules each containing 100 mg of 8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-methyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one.

Similar ampoules containing solutions suitable for parenteral administration may be prepared by proceeding in a similar manner but replacing the 8-(*N*-benzyl-*N*-phenylcarbamoyl-3-methyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one by another compound of the general formula shown in Figure I.

Composition Example 2

A solution suitable for parenteral administration was prepared from the following ingredients:—

3-(2-chloroethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one	1.0 g
dimethyl sulphoxide	10 ml
Arachis oil	90 ml

by dissolving the 3-(2-chloroethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one in the dimethyl sulphoxide and adding the arachis oil. The resulting solution was divided, under aseptic conditions, into ampoules at an amount of 10 ml per ampoule. The ampoules were sealed, to give 10 ampoules each containing 100 mg of 3-(2-chloroethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

Similar ampoules containing solutions suitable for parenteral administration may be prepared by proceeding in a similar manner but replacing the 3-(2-chloroethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-

Composition Example 3

Capsules suitable for oral administration were prepared by placing 3-(2-chl roethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one into gelatin shells of number 2 siz at a rate of 10 mg per capsule.

Similar capsules may be prepared by using another compound of the general formula shown in Figure I or any other conveniently sized capsule shells.

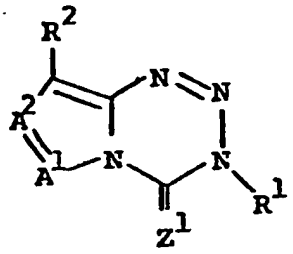


Fig. I

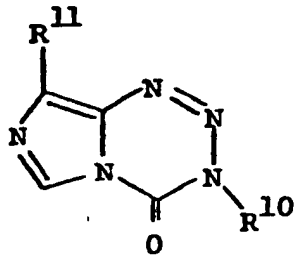


Fig. II

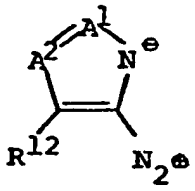


Fig. III

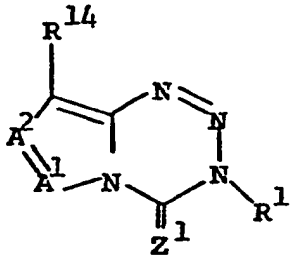


Fig. V

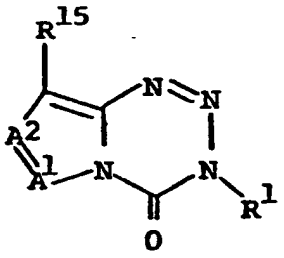


Fig. VI

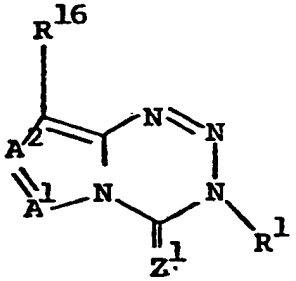
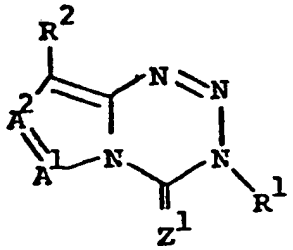


Fig. VII

Claims

1. Tetrazine derivatives of the general formula:



[wherein R^1 represents a cycloalkyl group containing 3 to 8 carbon atoms, or a straight- or branched-chain alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such alkyl, alkenyl or alkynyl group b ing unsubstituted or substituted by from on to three substituents selected from halogen atoms, straight- or branched-chain alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, A_1 represents a nitrogen atom or a group —CR^3= wherein R^3 represents a hydrogen atom or a substituent R^4 wherein R^4 represents a halogen atom, or a straight- or branched-chain alkyl or alkenyl group, containing up to 6 carbon atoms, which may carry up to 3 substituents selected from halogen atoms, optionally substituted phenyl groups, straight- or branched-chain alkoxy, alkylthio and alkylsulphonyl groups

cyano, hydroxy, nitro or optionally substituted phenoxy group, or a group of the formula —COR^5 (wherein R^5 represents an alkyl or alkoxy group of up to 4 carbon atoms, a hydroxy group, or an optionally substitut d phenyl group) or an alkanoylamino group containing up to 6 carbon atoms, or R^4 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$ or —$CZ^2NR^7R^8$ (wherein *n* represents 0, 1 or 2, R^6 represents a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, a cycloalkyl group containing 3 to 8 carbon atoms or an optionally substituted phenyl group R^7 and R^8, which may be the same or different, each represents a hydrogen atom or a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent or a cycloalkyl group containing 3 to 8 carbon atoms or an optionally substituted phenyl group or the group —NR^7R^8 represents a heterocyclic group, and Z^2 represents an oxygen or sulphur atom), A^2 represents a nitrogen atom or, when A^1 represents a nitrogen atom, A^2 represents a nitrogen atom or a group —CR^3= wherein R^3 is as hereinbefore defined, Z^1 represents an oxygen or sulphur atom, and R^2 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$, —$CSNR^7R^8$, —$CONR^7R^9$ or —CZ^2NHNO_2, wherein *n*, R^6, R^7, R^8 and Z^2 are as hereinbefore defined, and the group —NR^7R^9 represents a heterocyclic group, or R^7 is as hereinbefore defined and R^9 represents a straight- or branched-chain alkyl or alkenyl group containing up to 4 carbon atoms which carries an optionally substituted phenyl substituent, or an optionally substituted phenyl group or, when A^1 represents a nitrogen atom or a group —CR^4= wherein R^4 is as hereinbefore defined and Z^1 and A^2 are as hereinbefore defined or, when A^1 represents a group —CH= and Z^1 represents a sulphur atom and A^2 is as hereinbefore defined, R^2 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$, —$CZ^2NR^7R^8$ or CZ^2NHNO_2 wherein *n*, R^6, R^7, R^8 and Z^2 are as hereinbefore defined] and, when R^2 and/or R^3 represents a sulphamoyl or monosubstituted sulphamoyl group and/or R^3 represents a carboxy group, salts thereof.

2. Tetrazine derivatives according to claim 1 wherein R^1 is as defined in claim 1, R^2 represents a carbamoyl group optionally substituted on the nitrogen atom by one or two groups selected from straight- and branched-chain alkyl and alkenyl groups, containing up to 4 carbon atoms, and cycloalkyl groups containing 3 to 8 carbon atoms, A^1 represents a group —CR^4= wherein R^4 represents a straight- or branched-chain alkyl or alkenyl group containing up to 6 carbon atoms, which may carry up to 3 substituents selected from halogen atoms and optionally substituted phenyl groups or R^4 represents a cycloalkyl group containing 3 to 8 carbon atoms, A^2 represents a nitrogen atom, and Z^1 represents an oxygen atom.

3. Tetrazine derivatives according to claim 1, wherein R^1 is as defined in claim 1, R^2 represents a group of the formula —$S(O)_nR^6$ or —$SO_2NR^7R^8$, wherein *n* represents 0, 1 or 2, R^6 represents a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, a cycloalkyl group containing 3 to 8 carbon atoms, or an optionally substituted phenyl group, R^7 and R^8, which may be the same or different, each represents a hydrogen atom or a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, or a cycloalkyl group containing 3 to 8 carbon atoms, or an optionally substituted phenyl group, A^1 represents the group =CH—, A^2 represents a nitrogen atom, and Z^1 represents an oxygen atom.

4. Tetrazine derivatives according to claim 1 wherein R^1 is as defined in claim 1, R^2 represents a group of the formula —$S(O)_nR^6$, —$SO_2NR^7R^8$ or —$CZ^2NR^7R^8$ (wherein *n* represents 0, 1 or 2, R^6 represents a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, a cycloalkyl group containing 3 to 8 carbon atoms, or an optionally substituted phenyl group, R^7 and R^8, which may be the same or different, each represents a hydrogen atom or a straight- or branched-chain alkyl or alkenyl group, containing up to 4 carbon atoms, which may carry an optionally substituted phenyl substituent, or a cycloalkyl group containing 3 to 8 carbon atoms, or an optionally substituted phenyl group), A^1 represents a nitrogen atom, A^2 represents a group —CR^3=, wherein R^3 represents a hydrogen atom or a substituent R^4 as defined in claim 1, and Z^1 represents an oxygen or sulphur atom.

5. Tetrazine derivatives according to claim 1 wherein R^1 is as defined in claim 1, R^2 represents a carbamoyl group which carries on the nitrogen atom (i) two groups selected from optionally substituted phenyl groups and optionally substituted phenylalkyl groups; or (ii) one optionally substituted phenyl or optionally substituted phenylalkyl group; or (iii) one optionally substituted phenyl or optionally substituted phenylalkyl group and a straight- or branched-chain alkyl group containing from 1 to 4 carbon atoms, A^1 represents the group =CH—, A^2 represents a nitrogen atom, and Z^1 represents an oxygen atom.

6. Tetrazine derivatives according to any one of claims 1 to 5 in which the optional substituents on phenyl and phenoxy groups are selected from halogen atoms, alkyl and alkoxy groups containing up to 4 carbon atoms, and the nitro group.

7. Tetrazine derivatives according to any one of claims 1 to 6 in which the cycloalkyl group referred to is cyclohexyl.

8. Tetrazine derivatives according to claim 1 in which the heterocyclic group referred to is a 5-, 6-

nitrogen, oxygen and sulphur, and which may carry one or two straight- or branched-chain alkyl substituents each containing up to 4 carbon atoms.

9. Tetrazine derivatives according to claim 1 which have one or more of the following features:

(i) R^1 represents a methyl r 2-haloethyl group;

(ii) R^2 represents a group of the formula —SOR^6, —SO_2R^6, —$SO_2NR^7R^8$, —$CONR^7R^8$ or —$CONHNO_2$, wherein R^6, R^7 and R^8 are as defined in claim 1;

(iii) one of A^1 and A^2 represents a nitrogen atom and the other represents a group —CR^3= wherein R^3 is as defined in claim 1;

(iv) R^3 represents a substituent R^4 wherein R^4 represents an alkyl group containing up to 6 carbon atoms;

(v) A^2 represents a nitrogen atom;

(vi) Z^1 represents an oxygen atom; and/or

(vii) Z^2 represents an oxygen atom.

10. Tetrazine derivatives according to claim 9 wherein R^1 represents the 2-chloroethyl group.

11. Tetrazine derivatives according to claim 9 wherein R^2 represents a group —SOR^6, —SO_2R^6, —$SO_2NR^7R^8$, —$CONR^7R^8$ or —$CONHNO_2$ wherein R^6 represents an alkyl group containing up to 4 carbon atoms, R^7 represents a hydrogen atom or an alkyl group containing up to 4 carbon atoms, and R^8 represents a hydrogen atom, an alkyl group containing up to 4 carbon atoms, or a benzyl group optionally substituted by an alkoxy group.

12. Tetrazine derivatives according to claim 9 or 11 in which the alkyl group is methyl.

13. 8-Carbamoyl-3-(2-chloroethyl)-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

14. 3-(2-Chloroethyl)-6-methyl-8-sulphamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

15. 3-(2-Chloroethyl)-8-dimethylsulphamoyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

16. 3-(2-Chloroethyl)-8-(dimethylcarbamoyl)-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one.

17. 3-(2-Chloroethyl)-8-(*N*,*N*-dimethylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

18. 3-(2-Chloroethyl)-8-(*N*-methylsulphamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

19. 3-(2-Chloroethyl)-8-sulphamoyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazine-4-one.

20. 3-(2-Chloroethyl)-6-methyl-8-methylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

21. 8-(*N*-Benzylcarbamoyl)-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

22. 3-(2-Chloroethyl)-8-methylsulphonyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one.

23. 3-Methyl-8-methylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

24. 3-(2-Chloroethyl)-8-[*N*-(4-methoxybenzyl)sulphamoyl]-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

25. 8-Carbamoyl-3-(2-chloroethyl)-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one.

26. 3-(2-Chloroethyl)-8-piperidinocarbonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

27. 6-Butyl-8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

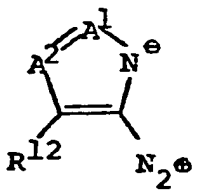
28. 8-Carbamoyl-3-(2-chloroethyl)-6-propyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

29. 8-Carbamoyl-3-(2-chloroethyl)-6-ethyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

30. 3-(2-Chloroethyl)-8-(*N*-nitrocarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

31. 8-(*N*-Benzyl-*N*-phenylcarbamoyl)-3-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 8-[*N*-benzyl-*N*-(4-methoxybenzyl)carbamoyl]-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-8-[*N*-(4-methoxybenzyl)-*N*-phenylcarbamoyl]-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-8-(*N*-phenylcarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 8-(*N*-benzyl-*N*-phenylcarbamoyl)-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-8-(*N*-methyl-*N*-phenylcarbamoyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 8-carbamoyl-3-methyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, 8-carbamoyl-3-(2-chloroethyl)-6-cyclohexyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 8-carbamoyl-3-(2-chloroethyl)-6-phenethyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 6-benzyl-8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 8-carbamoyl-3-(2-chloroethyl)-6-isopropyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-8-(4-methoxybenzyl)sulphamoyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-methyl-8-methylsulphonyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-8-methylsulphonyl-[3*H*]-pyrazolo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-6-methyl-8-methylsulphinyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one, 3-(2-chloroethyl)-8-ethylsulphonyl-6-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one and 3-(2-chloroethyl)-6-methyl-8-propylsulphonyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one.

32. A process for th preparation of tetrazin derivatives of the general formula depicted in claim 1, wherein R^2 is other than a sulphamoyl, mono(optionally substituted phenyl)carbamoyl or mono(optionally substituted phenyl)thiocarbamoyl, nitrocarbamoyl or nitrothiocarbamoyl group, which comprises reacting a compound of the general formula:

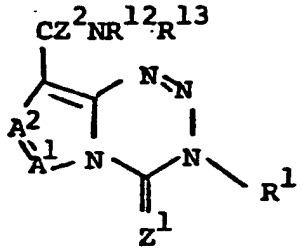


(wherein A^1 and A^2 are as defined in claim 1, and R^{12} represents a group within the definition of R^2 in claim 1 other than a sulphamoyl, mono(optionally substituted phenyl)carbamoyl or mono(optionally substituted phenyl)thiocarbamoyl, nitrocarbamoyl or nitrothiocarbamoyl group) with a compound of the general formula:

$$R^1NCZ^1$$

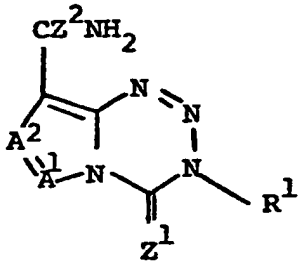
wherein R^1 and Z^1 are as defined in claim 1.

33. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1 wherein R^2 represents a mono(optionally substituted phenyl)carbamoyl or mono(optionally substituted phenyl)thiocarbamoyl group, and R^1, A^1, A^2 and Z^1 are as defined in claim 1, which comprises the debenzylation of a compound of the general formula:



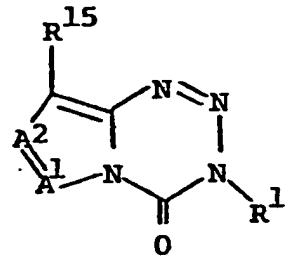
(wherein R^{12} represents an optionally substituted phenyl group, R^{13} represents an optionally substituted benzyl group and Z^2 is as defined in claim 1) by the application or adaptation of methods known *per se* for the replacement of optionally substituted benzyl groups by hydrogen atoms.

34. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1 wherein R^1, A^1, A^2 and Z^1 are as defined in claim 1 and R^2 represents a group of the formula $-CZ^2NHNO_2$, Z^2 being as defined in claim 1, which comprises the nitration of a corresponding compound of the general formula:



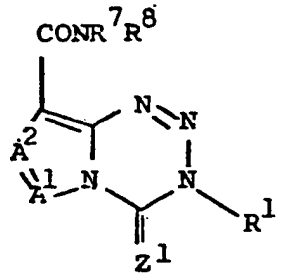
(wherein Z^2, R^1, A^1, A^2 and Z^1 are as defined in claim 1) to convert the grouping $-CZ^2NH_2$ to $-CZ^2NHNO_2$.

35. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1 wherein R^1, A^1, A^2 and R^2 are as defined in claim 1 and Z^1 represents a sulphur atom, which comprises reacting a compound of the general formula:



—$SO_2NR^7R^8$, —$CZ^2NR^7R^8$ or —CZ^2NHNO_2, R^6, R^7, R^8, *n* and Z^2 being as defined in claim 1) with phosphorus pentasulphide to convert the

36. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1, wherein R^1, A^1, A^2 and Z^1 are as defined in claim 1 and R^2 represents a group of the formula —$CSNR^7R^8$ wherein R^7 and R^8 are as defined in claim 1, which comprises reacting a corresponding compound of the general formula:



(wherein R^1, A^1, A^2, Z^1, R^7 and R^8 are as defined in claim 1) with phosphorus pentasulphide to convert the grouping —$CONR^7R^8$ to —$CSNR^7R^8$.

37. A process according to any of claims 32 to 36 wherein the tetrazine product obtained is a compound of the general formula depicted in claim 1 wherein R^2 and/or R^3 represents a sulphamoyl or monosubstituted sulphamoyl group and/or R^3 represents a carboxy group, and the product is converted by a method known *per se* into a salt, preferably an alkali metal salt.

38. Pharmaceutical compositions which comprise, as active ingredient, at least one tetrazine derivative as claimed in any one of claims 1 to 31 in association with a pharmaceutical carrier or coating.

39. Pharmaceutical compositions according to claim 38 substantially as hereinbefore described with especial reference to Composition Example 1, 2 or 3.

40. Tetrazine derivatives of the general formula depicted in claim 1, wherein R^1, R^2, A^1, A^2 and Z^1 are as defined in claim 1, for use in the treatment of malignant neoplasms such as carcinomas, melanomas, sarcomas, lymphomas and leukaemias.

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